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The Male Meiotic Phase in Five Species of Marsupials.

By

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With Plates 1 and 2.

INTRODUCTION.

THIS paper deals with the spermatogenesis of five species of marsupials including representatives of three families. The two species of Bettongia of the family Macropodidae have been studied in most detail. In the other forms little more has been done than to establish the number of chromosomes and the type of sex chromosomes.

I am indebted to Professor W. E. Agar for most of the material used and also for helpful criticism during the course of the work.

Bettongia Lesueuri (figs. 1–10, Pl. 1).

Material and Methods.—The material consisted of one lot of testicular tissue which had been fixed by Professor Agar. One-half of the material was fixed in Flemming, and the other half in Allen's Bouin without the urea and followed by anilin and bergamot oil as clearing agents. The Bouin material was much superior to that fixed in Flemming and was used exclusively. Most of the work was done with sections 10–15 μ thick, some mounted between coverslips so as to allow of the examination of the nuclei from both sides, and the remainder between slide and coverslip in the usual way. Heidenhain's iron haematoxylin was the standard stain used, mostly without counterstain, but in some cases in conjunction with eosin. In using iron haematoxylin it was found that very good differentiation could be obtained by replacing the iron alum ordinarily used for this purpose by a saturated aqueous solution of picric acid as recommended by Tuan (1930).

Number of Chromosomes.—Numerous counts of spermatogonial mitoses leave no doubt that the diploid number of chromosomes is twenty-two, or 20+XY in the male (figs. 1, 2, Pl. 1). This count has been checked from counts made during the first meiotic division. In the equatorial plates of the spermatogonial divisions the larger chromosomes are arranged in a ring enclosing several of the smaller chromosomes. In the case of Didelphys, which has the same number of chromosomes, Painter (1922) and Hoy and George (1929) have shown that normally the sex chromosomes alone are found in the centre of the plate, and the latter authors conclude that the occasional presence there of autosomes is due to an artifact. In Bettongia the X-chromosome could not be identified during spermatogonial divisions, but evidently lies in the central region of the equatorial plate, since first meiotic division figures show that it is amongst the smallest of the chromosomes. The Y is smaller and can be readily recognized. The arrangement of the chromosomes on the equatorial plate will be discussed later.

The Spermatogonia.—The resting spermatogonial nuclei have the chromatin distributed in the form of irregular faintly staining blocks connected by fine threads radiating out from the nucleolus. There is but one nucleolus present, and it stains densely with haematoxylin. A detailed description of the spermatogonia and of the primary spermatocyte will be given in connexion with an allied species, Bettongia penicillata, of which more favourable material was available.

Meiotic Phase.—The resting primary spermatocyte nuclei (fig. 3, Pl. 1) differ from those of the spermatogonia in that the chromatin blocks cannot be recognized. The nuclei are round and contain a large nucleolus. The chromatin is present as a faintly staining reticulum. In the leptotene nucleus (fig. 4, Pl. 1) the chromatin is in the form of fine tangled threads closely associated with a nucleolus. The process of syndesis could not be followed, but presumably takes place at the same time as the synizetic contraction. The chromatin threads which emerge from the contraction are comparatively thick and stain densely. In the late leptotene stage and throughout the pachytene there are two nucleoli present (fig. 5, Pl. 1). One of these is a karyosome and is the XY bivalent. It persists as a compact deeply staining body until the onset of the meiotic metaphase. The other structure is a plasmosome. It is usually closely associated with the karyosome and has a very variable staining reaction.

The pachytene cells then pass into a prolonged diffuse phase. The chromatin threads become scattered and lose their staining capacity, their outlines becoming irregular (fig. 6, Pl. 1). The nucleoli are not visibly affected during these changes (i.e. the XY bivalent does not share in the loss of staining capacity).

The chromosomes condense as thin threads which show an increasing affinity for stains. This marks the beginning of the diplotene (fig. 7, Pl. 1). The longer pairs of threads are loosely twisted about one another, and this condition is maintained as the threads contract. The contraction proceeds until the metaphase bivalents are formed, the nuclear membrane disappearing about this time. Of the eleven bivalents present ten are of double-thread type, while the eleventh is a compact rounded body (fig. 8, Pl. 1) which may show indications of a division into two. This structure is the XY bivalent or karyosome of the previous stage which has retained its compact form throughout the interval.

Owing to the irregular staining reaction of the plasmosome it was difficult to trace its behaviour during the later diplotene phase. It seems probable that it becomes reduced in size, and after the collapse of the nuclear membrane passes into the cytoplasm and finally disappears, as has been reported in other mammals by numerous observers (Allen, 1918; Painter, 1924*a*; Oguma, 1931). If so it could not be traced after passing into the cytoplasm.

In the ensuing division X and Y pass to opposite poles, becoming very elongated along the spindle fibre during their separation (figs. 9 and 10, Pl. 1). This condition is characteristic of the sex chromosomes of mammals, occurring to a greater or less extent in all forms in which the division has been observed. The first division thus gives the two types of secondary spermatocytes, the nuclei of which pass into a resting condition, the autosomes becoming diffuse. All the secondary spermatocytes retain a karyosome, presumably representing either the X- or the Y-chromosome.

During the pachytene stage of many, if not all spermatocytes, one or more small slightly chromatic bodies appear in the cytoplasm. They are probably of the same nature as the chromatoid bodies described by Agar (1923) in Petauroides. In later stages they stain only faintly, and their presence in the cytoplasm makes impossible the identification of the plasmosome after the disappearance of the nuclear membrane. They are distributed irregularly at the first meiotic division, but their subsequent history was not traced.

The secondary spermatocytes are very unfavourable objects for study on account of the small size of the nucleus and the tendency of the chromosomes to clump together.

Bettongia penicillata (figs. 11–17, Pl. 1, and figs. 18–20, Pl. 2).

Material and Methods.—The material of this species consisted of the testes of several males fixed in various ways. One lot of tissue fixed in Flemming by Professor Agar was particularly good, and most of the following account is based on it. In addition to iron haematoxylin, the methyl green + acid fuchsin combination was employed in order to study the nucleoli. The material fixed in corrosive acetic was used for this purpose.

Number of Chromosomes.—The diploid number of chromosomes as determined from spermatogonial divisions was found to be twenty-eight, or 26 + XY in the male, although the X-chromosome cannot be distinguished from the autosomes at this stage (figs. 11, 12, 13, Pl. 1). As will be seen later (figs. 19, 20, Pl. 2) the X-chromosome is J-shaped during the first meiotic division. If this is characteristic of it during spermatogonial divisions it is evident that it does not constantly lie in the centre of the equatorial plate. The count of twenty-eight is the highest yet recorded for any marsupial.

The Spermatogonia.—The chromatin in the nucleus of the resting spermatogonium is distributed in the form of faintly staining irregular blocks connected by fine strands which usually terminate in the nucleolus (fig. 14, Pl. 1). The blocks are evidently of the same nature as those described by Greenwood (1923) in Phascolarctus. They are present in approximately the diploid number, but an accurate count is difficult.

The nucleolus is a round or oval body lying close to the nuclear membrane. At this stage it stains deeply with haematoxylin, and its later behaviour shows that it is an amphinucleolus. During the spermatogonial prophase the chromatin blocks show an increasing affinity for stain, and the connecting strands finally disappear. While this is happening the nucleolus shows a decreasing affinity for stain, and reveals the presence of a number of deeply staining chromatic bodies lying in close contact with it (fig. 15, Pl. 1). It will be seen from fig. 15, Pl. 1, that these blocks are linked up by fine strands with the remaining chromatin of the nucleus. Throughout the prophase the chromatin blocks increase in chromaticity apparently at the expense of the nucleolus, which becomes progressively more faintly staining.

Fig. 16, Pl. 1, shows a later prophase in which the nucleolus is nearly free of chromatic material. The chromatin blocks have become more definitely chromosome shaped, and the connecting strands have disappeared. At the end of prophase the completely organized chromosomes lie close to the nuclear membrane and the nucleolus passes towards the centre (fig. 17, Pl. 1). It has regained its original shape, but in the case figured still retains a deeply staining body. It does not appear to be a constant feature of this stage, but in many cases a small chromosome is found in contact with the nucleolus after the others have separated completely.

The fact that the spermatogonial nucleolus is a plasmosome impregnated with chromatin agrees with the conditions in other marsupials (Jordan, 1911; Agar, 1923; Binder, 1927). In the case of Didelphys, Jordan has also described a similar extrusion of chromatin from the nucleolus during the prophase, but his account is not confirmed by Painter (1924*a*).

During the spermatogonial divisions the plasmosome can often be observed lying to one side of the equatorial plate. It is diminished in size and probably disappears later.

The Meiotic Phase.—This is similar in all essentials to that of Bettongia lesueuri. The nucleus of the resting spermatocyte (fig. 18, Pl. 2) is round and contains a large nucleolus, the structure of which is clearly shown in methyl green + acid fuchsin preparations. It then stains as a plasmosome, with an outer border of chromatic material connected by fine strands to the chromatin reticulum of the nucleus. The chromatin seems to gather on the surface of the plasmosome rather than to become incorporated into its structure.

In early leptotene stages the plasmosome becomes quite free of chromatin and often lies clear of the contracting threads. It could be easily traced through to pachytene stages at which there are two nucleoli, a karyosome and a plasmosome, which constantly lie close together as in Bettongia lesueuri. The fact that in Bettongia the pachytene plasmosome is identical with that of the leptotene conflicts with Agar's interpretation of the situation in Petauroides. In this animal there are often two plasmosomes in the pachytene nucleus, one of irregular shape in contact with the karyosome, the other of more regular outline and quite separate from the karyosome. Agar suggested that the latter plasmosome is persistent from the leptotene stage, the one in contact with the karyosome being a new one formed out of the substance of the XY bivalent (i.e. the karyosome). The similarity existing between Bettongia and Petauroides in the arrangement of the karyosome and a plasmosome in the pachytene nucleus suggests that the plasmosomes in question are corresponding structures, that is, the plasmosome lying close to the pachytene karyosome in Petauroides has persisted from the leptotene and is not an extrusion from the karyosome. On the other hand, the plasmosome in Bettongia rarely shows the variations in shape seen in the plasmosome occupying the corresponding position in Petauroides, but maintains a round or oval shape, and in this respect more closely resembles the other plasmosome in Petauroides.

The first meiotic division is differential for the sex chromosomes. The separation of X and Y precedes that of the autosomal bivalents. From figs. 19, 20, Pl. 2, it will be seen that the X-chromosome is J-shaped, the spindle fibre attachment being atelomitic. These figures also illustrate the fact that the sex chromosomes do not necessarily lie in the centre of the equatorial plate. At telophase they are typically found in the centre of their respective group of chromosomes. This is due to the early separation of the sex chromosomes.

Setonyx brachyurus (figs. 21–4, Pl. 2).

Material.—This marsupial has a limited distribution on the south-western coast of Australia, and I am indebted to Mr. G. Bourne of the University of Western Australia for the fixation of some of the best material. All the material was fixed in Allen's Bouin without urea.

Number of Chromosomes.—Spermatogonial divisions were very numerous in the testes of a young male. Equatorial plates consistently showed twenty-two chromosomes, and the presence of X- and Y-chromosomes was confirmed from meiotic divisions. The chromosomal formula for the male is therefore 20+XY (figs. 21, 22, Pl. 2).

The separation of the sex chromosomes takes place as in other forms (fig. 23, Pl. 2).

Fig. 24, Pl. 2, shows the chromosomes of a giant spermatogonium. The microtome knife has displaced a few chromosomes to the right, and farther over are drawn two groups from adjacent sections. The tendency for the small chromosomes to occupy the central region of the plate is very evident. Eighty chromosomes could be counted, the cell being evidently octoploid. Polyploid cells have been frequently reported from vertebrate tissues. Painter (1922, 1923) has recorded the presence of giant germ-cells in the lizard, opossum, and man. Hoy and George (1929) found a single 8 in. cell in the spleen of an opossum.

Petaurus breviceps (figs. 25–8, Pl. 2).

Material.—Material was available from two males, the fixative being in one case Bouin and in the other Allen's Bouin without urea.

Number of Chromosomes.—Counts were made from the spermatogonial metaphase plates of the two specimens, the diploid number being twenty-two (figs. 25, 26, 27, Pl. 2). The Y-chromosome is easily distinguished on account of its small size and position in the centre of the plate. The chromosome constitution of the male is therefore 20+XY. No attempt has been made to follow in detail the stages leading up to the meiotic divisions. Fig. 28, Pl. 2, shows a side view of the anaphase of the first division, the sex chromosomes behaving as usual.

Isoodon obesulus (figs. 29–33, Pl. 2).

Material.—Material was fixed in Flemming and in Allen's Bouin, the former method giving the better results.

Number of Chromosomes.—Spermatogonial divisions (figs. 29, 30, 31, Pl. 2) show that the diploid number of chromosomes is fourteen, and this count has been confirmed from first meiotic division figures which clearly show the seven bivalents (figs. 32, 33, Pl. 2). The sex chromosomes do not differ greatly from the smaller autosomes as regards size, nor are they so elongated as is usual at this stage. They have been identified at the first meiotic division as those which pass to the poles of the spindle in advance of the other chromosomes (figs. 32, 33, Pl. 2). Compared with the condition in most marsupials, the Y-chromosome is large in Isoodon.

During the spermatogonial divisions a plasmosome can usually be identified lying in the cytoplasm to one side of the equatorial plate (figs. 29, 30, Pl. 2).

CHROMOSOME ARRANGEMENT.

In the description of the spermatogonia of Bettongia lesueuri the arrangement of the chromosomes has been described, and reference has been made to the condition in Didelphys in which the sex chromosomes are isolated within a ring of autosomes. A similar isolation of the sex elements is seen in several Australian marsupials. Greenwood (1923) has described this condition in Phascolarctus, Sarcophilus, and Dasyurus, and it is also evident in Trichosurus and Potorous (Altmann and Ellery, 1925). In each of these cases the sex chromosomes are distinctly smaller than any of the autosomes, and their position in the centre of the equatorial plate is probably due simply to their small size and not to any peculiar property of sex chromosomes. This is indicated by the fact that in certain eutherian mammals in which sex chromosomes have been identified the X-chromosome is large and lies at the periphery of the plate. This condition is well shown in the dog (Minouchi, 1928). In these cases the central region is occupied by many small chromosomes, among which the Y can sometimes be identified (Painter, 1924, *b* and *c*). The tendency for small chromosomes to pass to the centre is shown also by Kuwada's (1929) work on chromosome arrangement.

CHROMOSOME NUMBERS.

Chromosome counts have now been made in sixteen species of marsupials, including representatives of six families. In the following table the number opposite each species represents the diploid number of chromosomes including X and Y.

The classification adopted is that put forward by Wood Jones (1923).

A. DIDACTYLA.			
1. Didelphidae	Didelphys virginiana	22	Painter, 1922.
2. Dasyuridae	Dasyurus maculatus	14	Greenwood, 1923.
	Sarcophilus ursinus	14	Ibid.
B. SYNDACTYLA.			
1. Peramelidae	Isoodon obesulus	14	This paper.
2. Phalangeridae	Pseudochirus peregrinus	20	Altmann and Ellery, 1925.
	Petauroides volans	22	Agar, 1923.
	Phascolarctus cinereus	16	Greenwood, 1923.
	Petaurus breviceps	22	This paper.
	Trichosurus vulpecula	20	Altmann and Ellery, 1925.

3. Macropodidae	Bettongia lesueuri	22	This paper.
	Bettongia penicillata	28	This paper.
	Potorous tridactyla	12	Altmann and Ellery, 1925.
	Macropus ualabatus	12	Agar, 1923.
	Macropus giganteus	22	Binder, 1927.
	Setonyx brachyurus	22	This paper.
4. Phascolomyidae	Phascolomys mitchelli	14	Altmann and Ellery, 1925.

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EXPLANATION OF PLATES 1 AND 2.

Except where otherwise stated figures are drawings of haematoxylin stained material.

REFERENCE LETTERS.

X, X-chromosome; *Y*, Y-chromosome; *XY*, XY bivalent; *pl.*, plasmosome.

PLATE 1.

Bettongia lesueuri (figs. 1–10)

Figs. 1–2.—Spermatogonial metaphase showing twenty-two chromosomes.

Fig. 3.—Resting nucleus of primary spermatocyte.

Fig. 4.—Leptotene nucleus.

Fig. 5.—Early pachytene showing karyosome and plasmosome.

Fig. 6.—Late pachytene (diffuse stage).

Fig. 7.—Diplotene.

Fig. 8.—Diakinesis showing eleven bivalents, including the compact XY.

Figs. 9–10.—First Meiotic division showing the separation of X and Y.

Bettongia penicillata (figs. 11–17).

Figs. 11–13.—Spermatogonial metaphase showing twenty-eight chromosomes.

Fig. 14.—Resting nucleus of spermatogonium.

Figs. 15–16.—Stages of prophase showing extrusion of chromatin from the nucleolus.

Fig. 17.—Late prophase.

PLATE 2.

Bettongia penicillata (figs. 18–20)

Fig. 18.—Resting nucleus of primary spermatocyte (methyl green and acid fuchsin staining).

Figs. 19–20.—First meiotic division showing sex chromosomes.

Setonyx brachyurus (figs. 21–4).

Figs. 21–2.—Spermatogonial metaphase showing twenty-two chromosomes.

Fig. 23.—First meiotic division.

Fig. 24.—Chromosomes of giant spermatogonium. To the right are shown chromosomes from adjacent sections.

Petaurus breviceps (figs. 25–8).

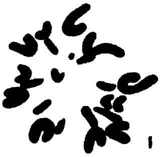
Figs. 25–7.—Spermatogonial metaphase (twenty-two chromosomes).

Fig. 28.—First meiotic division.

Isoodon obesulus (figs. 29–33).

Figs. 29–31.—Spermatogonial metaphase showing fourteen chromosomes. Figs. 29–30 also show the plasmosome.

Figs. 32–3.—First meiotic division showing sex chromosomes and six other bivalents.



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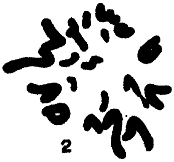

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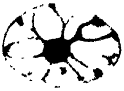

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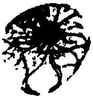

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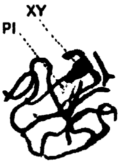

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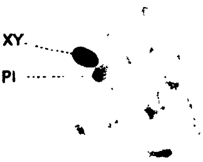

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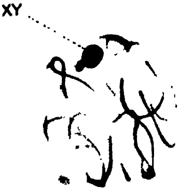

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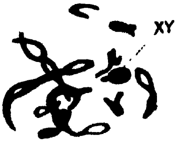

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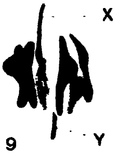

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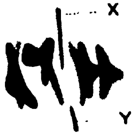

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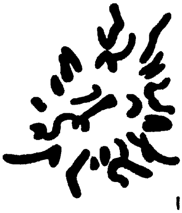

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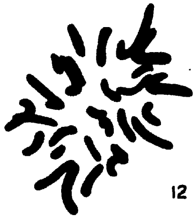

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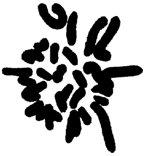

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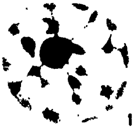

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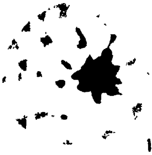

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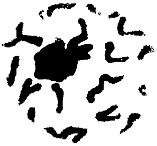

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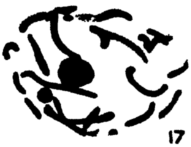

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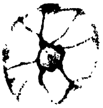
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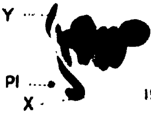

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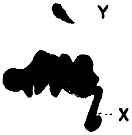

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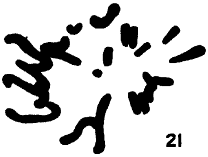
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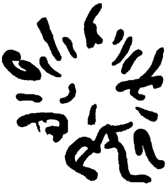
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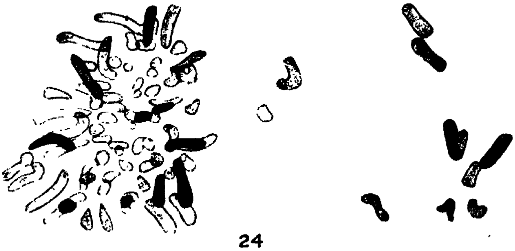
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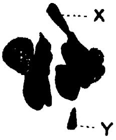

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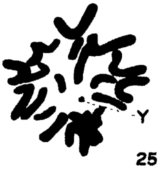

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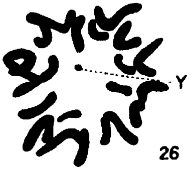

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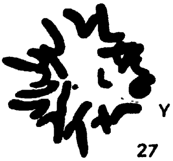

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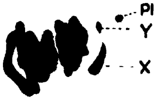

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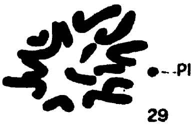

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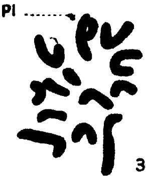

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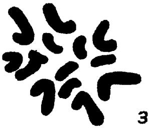
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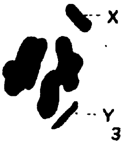

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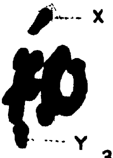

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F. H. Drummond, del.

The Miracidium of Heronimus chelydrae MacCallum.

By

James E. Lynch.

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With Plates 3 and 4 and 2 Text-figures.

INTRODUCTION.

IN August 1930 nine individuals of the snapping turtle, Chelydra serpentina, were taken from Stillwater Creek, Stillwater, Oklahoma, and examined for parasites. Seven of the nine turtles were infected with Heronimus chelydrae MacCallum, each harbouring from one to sixteen of these worms in the lungs. A total of forty-seven of the flukes was obtained.

As the writer was just on the point of moving from Stillwater, a prolonged and careful study of the live miracidia was impossible. The turtles were all killed within a week, and perhaps ten hours were spent studying the live miracidia, and in making notes, measurements, and sketches. A large number of miracidia was preserved in various ways and retained for subsequent investigation.

The miracidia were obtained by teasing the worms with needles in dishes of tap-water, and releasing the large mature eggs from terminal portions of the uterus. The eggs are thin-shelled, non-operculate, and of large size, averaging 256 by 164 microns. They promptly hatched large actively swimming miracidia. An interesting fact in connexion with the eggs is that the thin membranous shell enlarges as the miracidium grows; the more advanced the stage of development of the miracidium, the larger the egg.

The miracidia did not live long either in tap or distilled water. In from fifteen to thirty minutes after emerging from the egg the ectodermal cells swelled enormously, sometimes from ten to twenty times their original thickness, the rate of movement gradually diminished, and the organisms quickly died and disintegrated. The extremely short life of the hatched miracidia was probably abnormal, and due to the high temperatures prevailing in Oklahoma at the time. The daily maxima ranged from 100° to 106° F. in the shade, and there were no facilities available for lowering the temperature of the slides or dishes in which the miracidia were being observed.

The miracidia were preserved in a fair state of extension by quickly discharging them from a pipette into hot fixatives such as Bouin's, Schaudinn's, and acetic sublimate. Specimens for whole mounts were stained in alum cochineal, borax carmine, and iron-alum haematoxylin. The last, strongly destained, gave the most useful results. Sections were stained with Delafield's or iron-alum haematoxylin, and counterstained with erythrosin.

Cell boundaries were demonstrated by the following method: the miracidia were expelled from a pipette into a test-tube of hot (65–70° C.) 0·5 per cent. silver-nitrate solution. As soon as they had settled to the bottom the silver-nitrate solution was withdrawn. They were washed rapidly in three changes of distilled water, then placed in a Syracuse watch-dish in the sunlight for from three to ten minutes. As soon as the cell boundaries were sharply outlined the miracidia were removed from the sunlight, washed again in several changes of distilled water, and either preserved in glycerine (by placing them in 10 per cent. glycerine which was allowed to concentrate by evaporation), or dehydrated in graded alcohols, cleared, and mounted in balsam.

Morphology of the Miracidium.

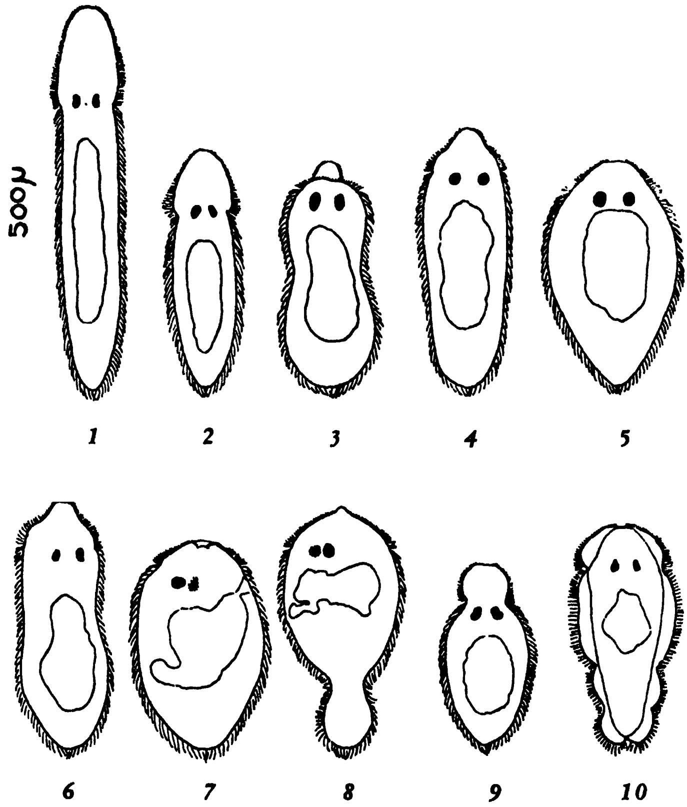
Shape.—The living miracidia have a plump cigar shape, with a conical anterior end and a rounded posterior end. In well-extended individuals the thickest part of the body is at the level of, or slightly posterior to, the eyes. Behind this point the animals taper gently to the bluntly rounded posterior end. In contracted specimens the thickest part of the body is near the middle.

The miracidia are extensible and contractile, and are capable of constricting the body at various points, consequently the length and proportions vary accordingly (Text-fig. A). The average dimensions of twenty preserved specimens selected at random were 300 (265–360) microns in length, 130 (100–155) microns in breadth, and 131 (100–150) microns in dorso-ventral thickness. Living specimens are capable of great elongation, and the larger ones sometimes stretch to a length of 500 microns.

The groove between the first and second tiers of cells, at the level of, or just anterior to, the eyes, is conspicuous and tends to set off a conical 'cephalic' region (Kopfkegel) which in markedly elongated swimming individuals may assume a greater diameter than the body posterior to the groove, and look remarkably head-like as seen with low magnifications. Often the anterior half of this conical segment is contracted into the form of a papilla, at the apex of which is the circular area (terebratorium of Reisinger) containing the pores of various glands. This projection may have a length of 40 microns, or it may not be delimited from the rest of the cephalic segment at all, except by its shorter, motionless cilia. Often the cephalic region is hemispherical, and the anterior area of glandular openings is withdrawn slightly beneath the surface. It is obvious that in this miracidium there is no permanent rostrum. The anterior half of the cephalic region may temporarily assume a narrower and cone-like shape, the result of muscular contractions, or there may be no trace of such a head papilla. The terebratorium itself cannot be protruded far enough to form a rostrum-like structure.

Cilia.—The surface of the miracidium is covered with closely set cilia. On preserved specimens the cilia posterior to the first tier of cells measure 8 to 11 microns, appearing slightly shorter in the anterior region of the body. On the cephalic cone (first tier of cells) they are about 10 microns in length at the posterior border, decrease to about 3 microns at the middle of the cephalic region, and to about 1·5 microns at the border of the terebratorium. The cilia of the anterior half of the cephalic cone are short, stiff, and motionless.

Terebratorium.—Although the 'rostrum', 'oral cone', 'apical papilla', or 'head papilla', of various authors is a structure which in many miracidia has no independent existence, and is formed by the temporary alterations in shape of the anterior part of the cephalic region, the area at the anterior



TEXT-FIG. A.

Outline drawings of the miracidia of Heronimus chelydrae to show temporary variations in shape and proportion. Nos. 1, 3, 4, and 10 are free-hand sketches of living miracidia; the remainder are camera lucidia outlines of preserved specimens. Nos. 7 and 8 are lateral views. The irregular outline within the miracidia represents the area of germ-balls. No. 10 depicts a moribund individual in which the epithelial cells have begun to swell. ×48.

end perforated by glandular pores is a structure common to all. Only Reisinger, to the best knowledge of the writer, has given it a name. Consequently his term, terebratorium, will be adopted in this paper for the non-ciliated, cuticularized area at the anterior end of the miracidium, perforated by pores through which the various glands discharge their secretions.

In the miracidium of Heronimus chelydrae the terebratorium is an area roughly elliptical in shape, its longer axis being transverse. It averages 21 by 15 microns, but the dimensions and proportions are variable, depending on the state of extension or retraction of the anterior end. In many specimens the terebratorium is practically circular in outline, except for the small out-pocketing on each side where the pores of the cephalic glands are located. In silver preparations the impregnated boundaries separating the anterior epithelial cells extend to it, and their anterior borders make a ring surrounding the area. The borders of all the pores likewise become impregnated with precipitated silver.

In the middle of the terebratorium there is a large pore, 1 to 2 microns in diameter, the so-called mouth, whose significance will be discussed later. At each lateral border there is a pore about 2 microns in diameter, the outlet of a cephalic gland. Scattered about over the surface are numerous small pores of varying size, mostly about 0·5 micron in diameter, through which the secretions of the gland usually called the intestine apparently are discharged. It is probable that some or all of these small pores contain sensory end-organs of the anterior nerves. The appearance of this area impregnated with silver is shown in fig. 8, Pl. 3; stained with iron-alum haematoxylin in fig. 7, Pl. 3.

In connexion with the conspicuous apical pores of the miracidium of Heronimus it is interesting to recall the observations of earlier investigators. Coe, in 1896, described a 'mouth' at the apex of the 'Kopfpapille' in the miracidium of Fasciola hepatica, and a group of pores immediately to each side of the mouth which he interpreted as openings of the cephalic glands. Ortmann (1908) failed to find a mouth in the miracidium of Fasciola, and described the cephalic glands as opening at the base of the rostellum far more laterally than did Coe. He suggests that the numerous pores of Coe are only secretion droplets of the cephalic glands. Reisinger (1923) figures numerous pores on the 'Porenkuppel' of the miracidium of Schistosoma haematobium, but does not figure a 'mouth' or pores for the cephalic glands. He, too, believes that Coe was in error as regards the mouth of the Fasciola miracidium. The distribution of pores on the terebratorium of the miracidium of Heronimus suggests that Coe was right as regards the 'mouth' and the small pores, but that he overlooked the openings of the cephalic glands, which, as Ortmann states, probably open laterally to Coe's group of small pores. It seems obvious that the last word on the glands and the terebratorium of the miracidium of Fasciola hepatica remains to be said.

Epithelial Cells.—Since hundreds of miracidia were prepared by silver impregnation, it was possible to make an accurate count of the number of epithelial cells. It was found that the cells always occur in four tiers, and that usually the anterior tier has six cells; the second, nine; the third, four; and the fourth, two.

However, the total of twenty-one epithelial cells occurred only in 44 per cent. of the miracidia. Table No. 1 shows the variations in the number of cells in the individual.

TABLE No. 1. Variations in the Number of Epithelial Cells of Miracidia.

Number of Epithelial Cells.	*Number of Individuals.*
16	1
17	0
18	5
19	18
20	31
21	44
22	1
	100

Some previous counts (Barlow, 1925; Mathias, 1925; Van Haitsma, 1930; Price, 1931; Sinitsin, 1931) indicate no variability in the number of epithelial cells, nor in the number of cells in a tier. However, Thomas (1883) and Coe (1896) mention a variability in the number and arrangement of the epithelial cells. The present study has demonstrated that in Heronimus chelydrae not only the total number of epithelial cells in the miracidium, but also the number of cells in any one tier is variable. This assertion is based upon counts of the epithelial cells in one hundred silver-impregnated specimens. The number of epithelial cells for the individual ranges from sixteen to twenty-two. Table No. 2 shows the variations in the number of cells in each tier.

TABLE No. 2. Variations in the Number of Epithelial Cells in each Tier.

	Number of Epithelial Cells.	*Number of Individuals.*
First Tier. . .	4	4
	5	24
	6	72
		100
Second Tier . .	6	2
	7	2
	8	41
	9	53
	10	2
		100
Third Tier . .	3	4
	4	94
	5	1
	6	1
		100
Fourth Tier . .	1	6
	2	94
		100

The second tier of cells shows the most variability, not only in the number of cells, but also in their size and position. Often it contains triangular cells which fail to extend the entire length of the tier.

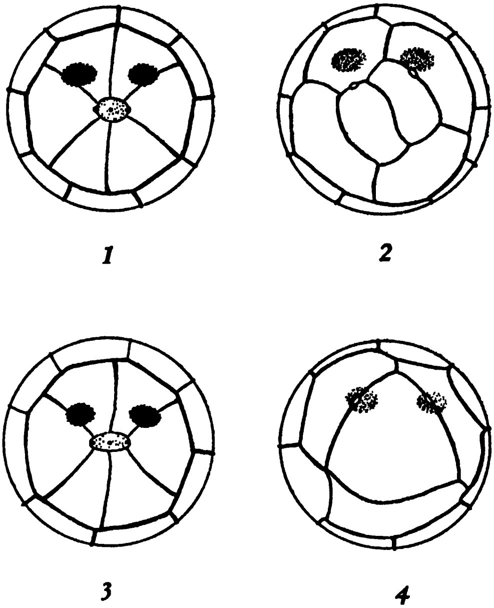
The impregnated areas between the epidermal cells are 3 to 6 microns in width. In unimpregnated sections these boundaries seem to be only 2 to 3 microns wide. The impregnated intercellular cement of the ring separating the first and second tiers of cells is perforated by numerous small holes from 1 to 1·5 micron in diameter. As seen in surface view (figs. 1, 2, Pl. 3) the intercellular cement bands present the appearance of two dark-brown, wavy or zigzag, but nearly parallel lines, separated by an area much lighter in colour. In cross-section (fig. 6, Pl. 3) the bands appear U-shaped, a vertical strip bounding the border of each epithelial cell, with a precipitation of silver at the bottom of the groove between the cells.

The appearances in this miracidium do not warrant the assumption that the subepithelial layer reaches the surface between the epithelial cells as has been reported for other miracidia (Coe, 1896; Price, 1931). It seems more likely that there is a definite cement substance between the cells in which the silver is deposited, and which separates the subepithelial layer from the surrounding medium.

The epithelial cells are extremely thin, and in individuals in which they are not swollen their thickness is only 1 to 1·5 micron. The nuclei are necessarily flat, less than a micron in thickness, and in surface views of whole mounts cannot be distinguished from the nuclei of the underlying subepithelial layer. However, they can be distinguished when seen in optical section at the border of the miracidium, or in paraffin sections. In moribund individuals whose epithelial cells have become greatly swollen they are easily seen. The nuclei are circular, 7 to 10 microns in diameter.

Subepithelial Layer.—The subepithelial layer (germinal epithelium of authors) is composed of large rounded cells, often binucleate. They are of variable size and shape, flattened at the base, averaging 10 microns high by 12 microns wide. The nuclei are approximately 5 microns in diameter. The majority of the subepithelial cells exhibit a distinct oxyphilic Nebenkern near the nucleus. Often they contain mitotic figures. For the most part they occur only as one cell-layer, but in certain regions, as at the points of attachment of the flame-cells, they are several cell-layers deep. At the posterior end these cells are much elongated, of irregular shapes, and extend anteriorly in such a way as to give the impression of a thick mass several cell-layers deep filling the posterior end of the body. Actually these cells form a pseudostratified epithelium in that region.

Muscular Layer.—The rather extensive changes of shape of which the miracidium is capable, as described above, indicate an effective muscular system. However, the muscular system is extremely obscure and cannot be demonstrated microscopically with perfect satisfaction. Structures which can be interpreted as muscle-fibres are found between the epithelial and the subepithelial layer. In longitudinal sections, or in optical sections of whole mounts, it can be seen that the epithelial and subepithelial cells are not contiguous, but are separated by a narrow space occupied by a series of lightly staining elliptical objects averaging 1 micron in length and 0·5 micron in height (fig. 9, Pl. 4, *c.m.*). They have the appearance of fine circular muscle-fibres, seen in cross-section. No nuclei can be detected in, or associated with, them. When a cross-section



TEXT-FIG. B.

Outline drawing of end views of silver-impregnated miracidia, balanced on end in glycerine. No. 1, an anterior; No. 2, a posterior view of an individual with twenty-one epithelial cells. Nos. 3 and 4, an individual with eighteen cells; cell-tiers 2, 3, and 4 having one less cell each than the miracidium figured in 1 and 2. × 150.

of the miracidium is examined, circular muscle-fibres cannot be distinguished with certainty. The space between the epithelial and subepithelial layers is occupied by an obscure meshwork, apparently formed by prolongations of the bases of subepithelial cells. Small dark points, 0·5 micron or less in diameter, are seen, spaced 2 to 4 microns apart; evidently longitudinal fibres in cross-section. These cannot be detected in whole mounts or in longitudinal sections. On the other hand, in some silver preparations in which the epithelial cells have accidently broken away from the subepithelial layer, the longitudinal fibres can be seen adhering to the inner surface of the epithelial cells. In the cephalic region the longitudinal fibres are slightly heavier and closer together. That these fibres, both circular and longitudinal, are muscle-fibres might well be doubted. They are extremely minute, and in most preparations appear only in cross-section as minute points. They give the impression of being processes of the subepithelial cells, although positive confirmation of this idea could not be obtained. They might, indeed, be processes or ridges of the outer epithelial cells. The fibres described above are the only differentiations in the body of the miracidium which in appearance and location seem capable of effecting the changes of form which the miracidium is able to accomplish. They are in the position of the muscle-fibres described in other miracidia (Leuckart, 1882, 1886–1901; Looss, 1892, 1886–1901; Ortmann, 1908; Reisinger, 1923).

Nervous System.—The cerebral ganglion consists of a quadrangular mass of fibrillar substance (fig. 9, Pl. 4, *f.b.*) bilobed in dorsal or ventral view, surrounded by several layers of closely packed nerve-cells. It is located dorsally, posterior to the cephalic cone, between the eyes and the anterior end of the germ-ball mass. The fibrillar portion of the brain averages 40 microns in breadth, 21 microns in dorso-ventral thickness, and 24 microns in length across the broadest part of either the right or left lobe. This portion of the brain is surrounded by from two to four layers of small nerve-cells, averaging 6·7 by 5·8 microns, with chromophobe cytoplasm. The nuclei average 4·2 microns in diameter, and practically every nucleus contains a clear vacuole (fig. 9, *n.c.*) in its centre.

From the anterior side of the fibrillar mass two nerves are given off which run forward toward the terebratorium between the ducts of the cephalic glands and the centrally located 'intestine'. Their precise terminations could not be determined, since they become indistinct a few microns from the terebratorium. It seems likely that they come into relation with some or all of the small pores of the terebratorium. From the sides of the brain two nerves arise which run laterally a short distance, then turn antero-laterally to end at the two lateral sense papillae.

Postero-laterally the brain sends off two nerves which run posteriorly and laterally along the sides of the germ-ball mass to merge with the body-wall near the middle of the animal. The arrangement of the three pairs of nerves is shown in fig. 9, *a.n.*, *l.n.*, *p.n.*

Sense Organs.—The eyes (fig. 9, Pl. 4) appear as two reniform black spots below and a short distance posterior to the junction of the first and second tiers of epithelial cells. They are located on the antero-dorsal border of the fibrillar part of the brain. The lens is extremely inconspicuous, but is occasionally evident in whole mounts. The average size of the pigmented masses, as seen from the dorsal side, in ten miracidia, is 17·3 by 13·1 microns for the right eye, and 18·6 by 16·6 microns for the left eye. The greater size of the left eye is easily discerned without recourse to measurement. In thin serial sections (fig. 3, Pl. 3) the eye is seen to consist of a mass of protoplasm, densely crowded with brown melanin granules, containing one or several nuclei. On the lateral surface is an inconspicuous lens which does not protrude far from the pigmented mass. It is evidently composed of soft material, for in a certain proportion of the eyes studied it appears collapsed. The lens of the eye is a biconvex disk, 7 to 9 microns in diameter, 3 to 6 microns thick.

In the groove between the cells of the first and second tiers there are a pair of sense papillae (anterior ducts, anterior papillae, mucoid secretion, lateral papillae, lateral processes, of authors) located laterally, one on each side (fig. 9, *s.p.*). Each papilla appears as a clear structure, approximately hemispherical, 2·5 to 3 microns high, 2·5 to 4 microns in diameter. In occasional individuals there is a much smaller papilla, either anterior or posterior to the larger one. Only a careful study of living miracidia would demonstrate whether or not these accessory papillae are constant and uniform structures. The sense papillae were overlooked by the writer when the living miracidia were being studied, but they are readily seen in serial sections or in whole mounts of individuals which lie on their dorsal or ventral surfaces. In addition to the two lateral papillae, the groove between the first and second tiers of cells contains an undetermined but small number of motionless bristles about 3 microns in length. Both sectioned material stained with haematoxylin, and whole mounts of silver-impregnated specimens, demonstrate that the intercellular cement between the cells of the first two tiers is perforated by a large number of small pores 0·5 to 1·5 micron in diameter, circular to oval in outline. Although the pores are arranged with some irregularity they show a tendency to be disposed in two more or less parallel rows. They are far too numerous to be regarded as the points of protrusion of the few scattered bristles. They undoubtedly represent a sense organ of some kind, perhaps the origin of sensory nerve-fibres connected with the lateral nerves, or of sensory neurones of the type described by Price (1931) in the miracidium of Schistosomatium douthitti.

Apical Gland.—The anterior gland, usually called the 'intestine', 'primitive gut', 'rhabdocoele gut', or 'rudimentary digestive tract' by writers on the Trematoda, is a granular, flask-shaped structure of extremely variable proportions, depending on the state of extension of the miracidium. In elongated animals it may extend far posteriorly to the eyes, ventrally to the brain. In individuals whose anterior end is contracted the 'intestine' may be shortened, broadened transversely, and may lie entirely in the anterior half of the cephalic, or preocular, region. In specimens of average extension it occupies about 20 per cent. of the body length, and is anterior to the brain. A dilated posterior portion comprises 55 per cent., a narrower anterior region 45 per cent. of the total length of the gland. The basophilic contents are coarsely granular. In the posterior part of the gland is a group of four nuclei.

A peculiarity of the anterior region is that it first divides into two portions, each of which divides again, so that it is formed of four limbs surrounding a central space. Each limb spreads out beneath the terebratorium and sends off numerous small processes to the surface (fig. 4, Pl. 3). Apparently each of these delicate processes terminates in a pore on the terebratorium, although the supposition could not be verified because of the intense staining of the cuticula of the terebratorium in sectioned miracidia. The shape and proportions of the limbs of the gland are moderately variable. In many individuals there are only three limbs instead of four.

Oxyphilic Cells.—Surrounded by the four limbs of this gland are two large, conspicuous, oxyphilic cells. Their proportions depend upon the state of contraction of the anterior end. In well-extended specimens the cells are about 32 microns in length, the fundus of the cells is about 8 microns in width, and the anterior region 5 to 6 microns in diameter (fig. 9, *o.c.*). The cytoplasm of the cells is granular and stains a brilliant red with erythrosin. The nuclei are 5 to 6 microns in diameter. The anterior ends of the two oxyphilic cells surround a small flask-like cavity opening in the middle of the terebratorium in a conspicuous pore, which in other kinds of miracidia has usually been designated the 'mouth'. The opening is 1 to 2 microns in diameter, the pouch-like space 6 to 7 microns in length and 2 to 3 microns in width at its broadest part. This cavity, opening at a pore in the centre of the terebratorium, seems obviously to be merely the duct or external aperture of the two glandular cells.

The writer can see no reason for designating any or all of the cells of this complex an intestine. The large saccate gland with the four nuclei obviously corresponds with the structure commonly called the intestine in most descriptions of miracidia. But in Heronimus its anterior region breaks up into four separate arms, each of which expands under the terebratorium and seemingly gives off capillary ducts to the pores of that structure. It is hard to homologize such an organ, a syncytial structure without lumen or mouth, with a digestive tube. It is plainly a gland whose secretions are poured out at the apical end of the animal. In most miracidia the intestine has been described as a solid structure without trace of lumen. Even in those in which it is multicellular (e.g. in Diplostomum flexicaudum, Van Haitsma, 1930) there is no lumen in the ordinary sense of the word. The so-called mouth is only a pore, without trace of oral or pharyngeal musculature. In agreement with the morphological character of this structure we might recall that no miracidium has ever been observed to ingest anything, although they have been observed to discharge granules (under pressure?) from the anterior opening of the 'intestine' (Leuckart, 1886–1901, 'Parasiten des Menschen', p. 257; Looss, ibid., p. 525).

The function of this organ is obviously glandular, not nutritive, a fact recognized by all recent students of miracidia, in spite of the fact that they cling to the old nomenclature as a matter of convenience. In the absence of any appropriate name for this organ, the writer would like to propose the term apical gland for the anterior gland usually termed the intestine by helminthologists.

Cephalic Glands.—The two cephalic glands (salivary glands, penetration glands, secretion glands, of authors) are ventral in position. They extend much farther posteriorly than the apical gland. Each consists of a posterior bulb-like portion and a long neck which curves laterally and follows the inner surface of the cephalic body-wall to end in a distinct pore on each lateral border of the terebratorium. Each gland is uninucleate. The posterior bulbous portion averages about 50 microns in length, 31 microns in breadth, and 22 microns in dorso-ventral thickness. The necks are 7 to 4 microns wide, 50 to 75 microns long. The two posterior saccate portions are often very closely appressed in the median line. These gland-cells stain feebly, and probably secrete a mucoid substance. The cephalic glands, in fixed individuals in an average state of elongation, extend 38 per cent. of the total body length. In many preserved and mounted specimens a rounded secretion droplet is evident at the excretory pore of one or both cephalic glands.

Excretory System.—The excretory system is composed of two large flame-cells, one anterior and one posterior, suspended from the dorsal body-wall, together with their tubules and associated cells. The anterior flame-cell is located just back of the brain in the forward half of the second tier of cells; the posterior flame-cell is behind the germ-ball mass, near the middle of the third tier of cells. In fixed material each flame-cell is seen to be composed of a large nucleated cap-cell about 20 microns in width and 10 to 15 microns in height, bearing a triangular tuft of cilia averaging 17 microns in length. In living specimens the undulating 'flame' is easily visible, and often much larger than indicated above; in fact in one live miracidium the 'flame' alone was 30 microns in length. Each tuft of cilia is surrounded by a funnel-shaped expansion of the end of the excretory tubule.

The flame-cells are suspended from the dorsal body-wall near the median line by a group of cells much resembling those of the subepithelial layer. The group forms a suspensorium (fig. 1, *s.f.c.*, Pl. 3), one or two cell-layers deep, to which the flame-cell adheres. The apex of the latter points ventrally, but may be deflected in any direction by the pressure of other structures.

The tubules of the excretory system are very hard to trace. The writer was unable to trace them in living miracidia. In whole mounts impregnated with silver they often are outlined for part of their length, and in sections they usually can be traced for varying distances, but it was impossible, in any one specimen, to trace the tubules from end to end. Their course, as interpreted from a number of specimens, each demonstrating some part of the excretory system, is as follows: from the apex of the anterior flame-cell proceeds a tubule which is highly convoluted in its proximal portion, but which continues posteriorly from its initial convolutions with only occasional kinks and loops, to open at the excretory pore, located dorso-laterally in the boundary between the epidermal cells of tiers three and four.

The tubule from the posterior flame-cell, after first coiling about a pair of adjacent large cells (fig. 9, Pl. 4, *a.e.c.*) proceeds with many loops and twists anteriorly almost to the level of the anterior flame-cell, then turns back and runs posteriorly to the excretory pore of the left side, which has a position similar to that of the right side.

The tubules of the excretory system are dorso-lateral in position. Near the external opening each dilates into a small excretory vesicle which often is collapsed or indistinct. The dimensions of the excretory pore average 5·5 by 3·5 microns. It is interesting to note that Coe (1896) and Price (1931) also found the excretory pores located between the epithelial cells, whereas Ortmann (1908), Mathias (1925), Van Haitsma (1930), and Sinitsin (1931) plainly figure the excretory tubules perforating the epithelial cells.

In the posterior end of the body are two large cells (fig. 9, *a.e.c*), averaging 20 microns in diameter, attached to the lateral body-walls. They are always located approximately at the level of the posterior flame-cell. The protonephridial tubule is adherent to them, curving about their peripheries. It was not possible to decide whether only the tubule of the posterior flame-cell or the tubules of both flame-cells adhere to them, although probably both tubules come into relation with them. Their cytoplasm is pale and vacuolated. It seems possible that these cells perform some excretory function, therefore they have been designated accessory excretory cells.

A study of sections first demonstrated that occasional individuals have more than two flame-cells. An examination of twenty-nine whole mounts in which the flame-cells were distinctly visible revealed five with three flame-cells and two with four flame-cells. The additional flame-cells were always in the posterior end. Their relation to the tubules of the other two flame-cells could not be determined.

Germ-balls.—The central part of the body of the miracidium is occupied by a mass of germ-balls (fig. 9, Pl. 4, *g.b.*). They are more or less ellipsoidal in shape, and are multicellular, or at least multinucleate. Cell boundaries almost never can be distinguished, although actually present, as can be seen in some specimens in which marked shrinkage has taken place. The outer layer of each germ-ball is a thin envelope of closely adherent, flattened, inconspicuous cells. The germ-balls at the anterior end are the largest, averaging 41 by 35 microns, and attaining a maximum size of 50 by 38 microns. Near the middle their average dimensions are 25 by 21 microns. At the posterior end the germ-balls, though small, are of variable size. Many are composed of only two or three cells. The smallest one measured was 11 by 7 microns. It can be seen in sections that the largest germ-balls have a small central lumen, which causes them to resemble a blastula, although the wall of the embryo is several cell-layers thick. The germ-ball mass is surrounded by a thin membranous sac, from which partitions pass inward to form a separate compartment for each embryo (fig. 9, *g.b.s.*). The membranes are protoplasmic, bearing flattened nuclei here and there. At the ventral side of the anterior end the envelope is anchored to the body-wall by a compact group of small cells (fig. 1, *a.s.*, Pl. 3) which is broadest at its point of attachment to the germ-ball sac, and which tapers to its insertion in the body-wall 7 to 15 microns back of the boundary between cell-tiers one and two. The dimensions of this structure vary with the extent of contraction or elongation of the miracidium. In an elongated individual the 'anchor' may stretch to 70 microns in length, and be only 12 microns in width posteriorly. In a contracted specimen the strand may be only 30 microns long and 27 microns wide at its posterior end. Anteriorly the strand always tapers to from 7 to 3 microns in diameter, especially after entering the layer of subepithelial cells. Its precise relations to the body-wall are obscure; apparently it is inserted on a ventral epithelial cell a short distance behind its anterior boundary. The nuclei of the anchoring strand are small, from 3 by 2 to 4 by 3 microns, and resemble the nuclei of the membranous sac and the partitions between the germ-balls.

At the posterior end of the germ-ball mass some of the smaller embryos form an appendage which turns dorsally and crowds against the dorsal body-wall (fig. 1, Pl. 3; Text-fig. A, 7, 8). There is no evidence that the germ-cells proliferate from this region. Strands or partitions of the membranous envelope fuse with the body-wall in the posterior end at various places. These lamellae from the envelope form a kind of diffuse parenchyma in the posterior end of the body between the group of germ-balls and the subepithelial cells.

SUMMARY AND DISCUSSION.

The miracidium of the monostome fluke, Heronimus chelydrae, described in this paper, is noteworthy on account of its large size, since it attains a maximum length of half a millimetre.

The epithelial cells of one hundred individuals were counted. The cells occur in four tiers. Although the number of cells in each tier, and the total number in each miracidium, presents a definite mode, nevertheless a notable range of variation has been shown to exist in each case. This variation is of interest in that it indicates the dubious value of the number and arrangement of the epithelial cells in determining family relationships or symmetry.

It is pointed out that most earlier workers have failed to distinguish between the cilia-free sensory and glandular area at the anterior end of a miracidium and the temporary conical constrictions of the apical region caused by muscular contraction. It is suggested that Reisinger's term terebratorium be adopted for the area of glandular orifices and, presumably, sensory end-organs, and that the term rostrum, or similar terms, be employed only in case the former is located on, or forms, a permanent papilla or snout-like differentiation of the anterior end.

Apparently Coe and the writer are the only persons who have studied the terebratorium in end view. The present study shows that there are three distinct types of openings in the terebratorium of the miracidium of Heronimus, and the conflicting descriptions of several investigators suggest that some other kinds of miracidia possess the same three types of pores. The large central pore, heretofore called a mouth, cannot be considered a mouth in the miracidium of Heronimus, where it obviously is the aperture of a gland. The minute pores scattered over the terebratorium probably represent glandular orifices, nerve terminations, or both. The openings of the cephalic glands are located laterally within the area of the terebratorium.

The structure usually designated an intestine is shown in the miracidium of Heronimus to be glandular and not to have the remotest morphological resemblance to a digestive tract. In this animal it seemingly does not even enter into relation with the aperture usually called the mouth. As its precise function is unknown it is given the non-committal name of apical gland in this paper.

Two large oxyphilic cells are described whose distal ends enclose a small cavity communicating with the outside through the central pore formerly termed the mouth.

The subepithelial muscle-layer of this miracidium is very poorly developed. It can be recognized as a thin layer of minute fibres between the ciliated epithelial cells and the subepithelial cells. The fibres, circular and longitudinal, appear to be extensions of the bases of the subepithelial cells.

Three pairs of large nerves arise from the brain. The anterior pair passes forward to the region of the terebratorium, and probably terminates in the pores of that organ. The lateral nerves terminate at the two lateral sense papillae. The unmistakable nature of the lateral nerves in the miracidium of Heronimus, and in that of Schistosoma haematobium as described by Reisinger, as well as the observations of Coe, Reisinger, Van Haitsma, Price, Sinitsin, and the writer on the sense papillae at the junction of the first two cell-tiers in various miracidia, would seem effectively to invalidate earlier interpretations of these papillae as drops of secretion issuing from 'lateral gland ducts' (i.e. lateral nerves), and of the lateral glands as well. The lateral glands (Faust, 1924), indeed, probably are only the nerve-cells surrounding the medullary fibrillar mass of the brain.

The excretory system of the Heronimus miracidium consists of one anterior and one posterior flame-cell, each with a separate capillary duct. However, a considerable proportion of the miracidia has one to two supernumerary flame-cells in the posterior end. Two large 'accessory excretory cells', right and left, situated at the posterior end of the body are associated with one or both excretory tubules.

The aggregate of germ-balls of the Heronimus miracidium is enclosed in a membranous cellular envelope, from which partitions pass centrally to enclose each germ-ball in its own compartment. The germ-ball sac is anchored to the anterior ventral body-wall by a conspicuous cord of cells. Posteriorly it is fastened to the body-wall by a number of delicate cellular membranes. A complete embryo sac, separating the germ-balls from the adjacent subepithelial layer has not been previously described.

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EXPLANATION OF PLATES 3 AND 4.

PLATE 3.

Drawn with camera lucida, except as otherwise noted.

Fig. 1.—Miracidium of Heronimus chelydrae. Lateral view of silver-impregnated specimen. Camera lucida drawing, slightly schematized. Excretory tubules were not seen in the specimen drawn, but were depicted as reconstructed from a study of a number of specimens. Abbreviations: *a.g.*, apical gland; *a.s.*, anterior anchoring strand of germ-ball sac; *c.g.*, cephalic gland; *c.g.d.*, duct of cephalic gland; *e.p.*, excretory pore; *f.c.*, flame-cell; *n.c.*, nerve-cells; *s.f.c.*, suspensor of flame-cell; *t.e.*, terebratorium. ×300.

Fig. 2.—Boundary between the first and second tiers of epithelial cells. Silver preparation, showing the numerous pores in the intercellular cement. Abbreviation: *s.p.*, sense papilla. ×1120.

Fig. 3.—Frontal section of an eye. ×720.

Fig. 4.—Apical gland, reconstructed from serial sections. ×480.

Fig. 5.—Drawing of a silver preparation to show the arrangement of the cell boundaries in a specimen with twenty-one epithelial cells. Note the different arrangement of the cells in tiers 1 and 2, with reference to the median sagittal plane, as compared with the specimens illustrated in Text-fig. A. ×108.

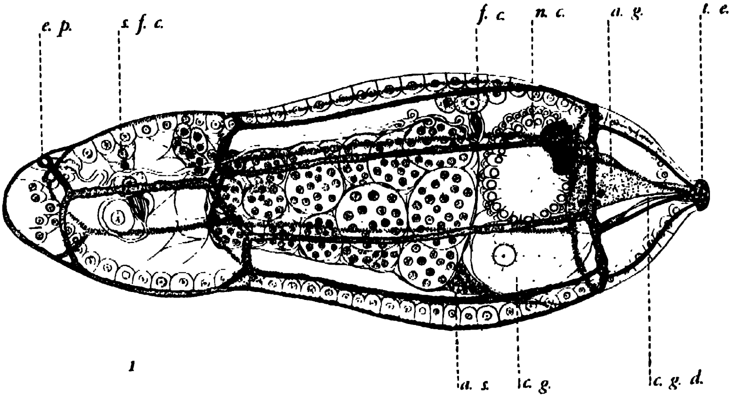
Fig. 6.—Cross-section of an epithelial cell in a silver preparation to show the structure of the impregnated intercellular cement in cross-section. The cell is slightly swollen. Abbreviation: *i.c.*, intercellular cement. ×1120.

Fig. 7.—Terebratorium, end view. Section from a specimen fixed in acetic sublimate and stained with iron-alum haematoxylin. ×1370.

Fig. 8.—Terebratorium, end view. Section from a silver preparation. Abbreviation: *c.g.d.*, duct of cephalic gland. ×1120.

PLATE 4.

Fig. 9.—Miracidium of Heronimus chelydrae. Schematic. Abbreviations: *a.e.c.*, accessory excretory cell; *a.g.*, apical gland; *a.n.*, anterior nerve; *c.b.*, boundary between epithelial cells; *c.g.*, cephalic gland; *c.g.d.*, neck of cephalic gland; *c.m.*, circular muscle-fibres; *e.p.*, excretory pore; *f.b.*, fibrillar part of brain; *f.c.*, flame-cell; *g.b.*, germ-ball; *g.b.s.*, germ-ball sac; *l.n.*, lateral nerve; *n.c.*, nerve-cells; *o.c.*, oxyphilic cells; *p.n.*, posterior nerve; *s.d.*, secretion droplet; *s.p.*, sensory papilla. ×530.



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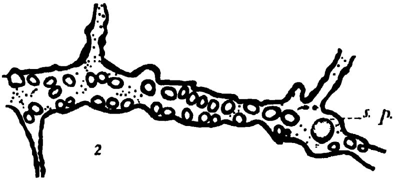

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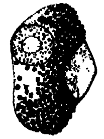

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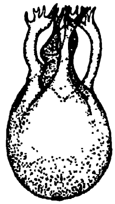

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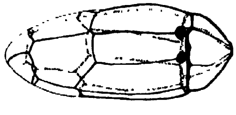

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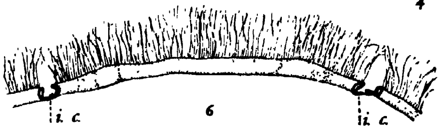

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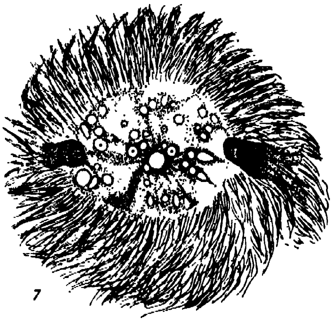

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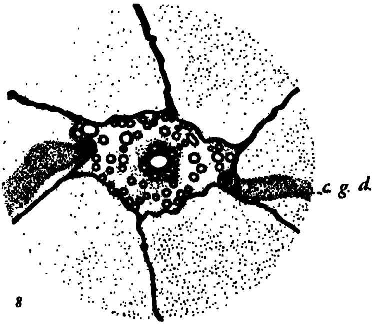

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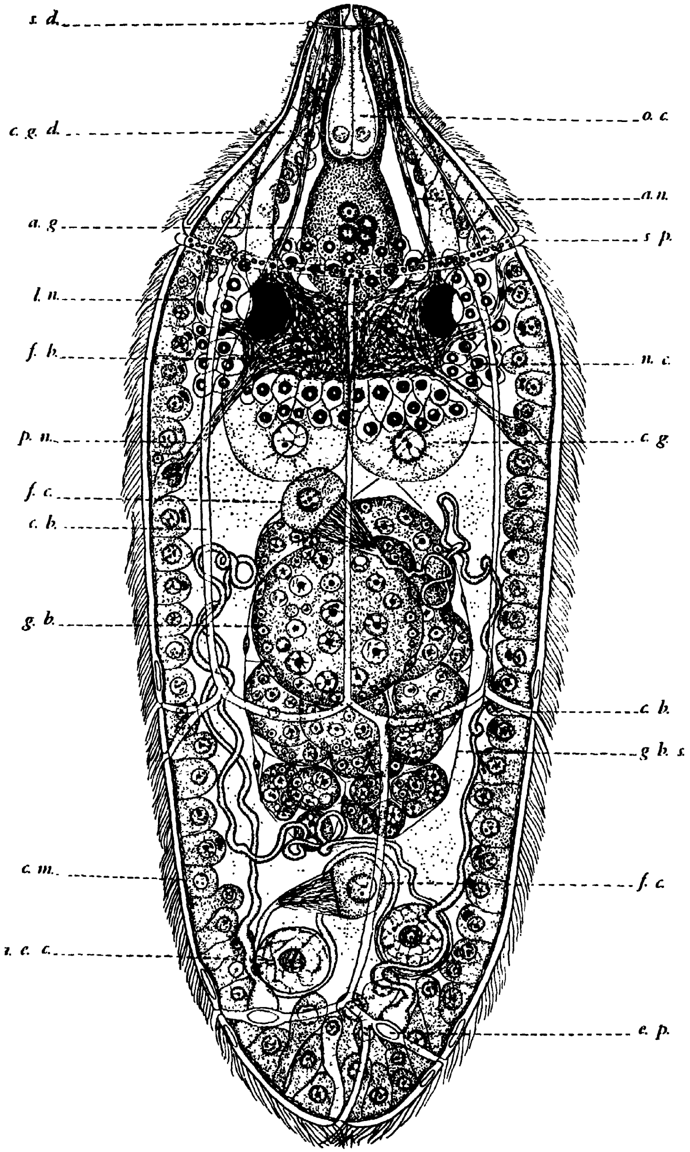

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9

On the Development of the Male Genitalia and the Efferent Genital Ducts in Lepidoptera.

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With 18 Text-figures.

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INTRODUCTION.

ONLY in a few groups of insects has the homology of the external genitalia been determined by investigations into the development of these organs.

During the concluding part of the last century two conflicting views were held regarding the nature of gonapophyses. According to Lacaze-Duthiers (1849–53), Grassi (1889), and Haase (1889) they are integumental outgrowths corresponding to the 'styloid' processes ('Griffel') which are present on the abdomen and inserted at the bases of the legs in such generalized insects as the Thysanura.

On the other hand, Weismann (1864), Kraepelin (1873), Dewitz (1875), Huxley (1877), and Cholodkovsky (1891 *a*) showed that the gonapophyses are comparable to the true ambulatory legs. In this connexion Kraepelin (1873), Dewitz (1875), and others described these appendages as arising from imaginal discs, and Wheeler (1893) showed that there exists a direct continuity of the embryonic appendages with the gonapophyses.

Zander (1900, 1901, 1903) actually described the development of the penis and the gonapophyses ('valvae') in the post-embryonic stages of Hymenoptera, Trichoptera, and Lepidoptera from paired rudiments or buds on the ninth abdominal segment. This formed a basis for later work on these lines, and his conclusions were largely confirmed in certain other insect orders. In this connexion may be mentioned the work of Muir (1915, 1918) and Singh-Pruthi (1924) on Coleoptera, Christophers and his collaborators (1922, 1926) on Diptera, Kershaw and Muir (1922), Singh-Pruthi (1924) and George (1929) on Homoptera and Zygoptera respectively.

Verson and Bisson (1896) also referred to the development of the male genitalia in Bombyx mori Linn. in their extensive memoir on the origin and nature of the efferent genital ducts in this species. Their conclusions, however, were criticized by Zander (1903), and his work, since then, is the only comprehensive account to which reference could be made for determining the homologies of the elements composing the insect genitalia.

It has also been realized that the mode of origin of the 'valvae' as described by Zander (1903) is open to question (Singh-Pruthi, 1924), and his conclusions differ from those of his predecessors (Verson and Bisson, 1896, and others). The development of the posterior abdominal segments and their appendages in Lepidoptera need further reinvestigation, because Zander's observations (1903) on these lines, though conclusive, were not confirmed by subsequent workers. It therefore is desirable that the whole problem should be revised in the light of existing knowledge of orders other than the Lepidoptera. With this in view I have studied the development of the male genitalia in the following Lepidoptera, viz. Hepialus lupulinus Linn., Pieris rapae Linn., Earias fabia Stoll., and Bombyx mori Linn., as representatives of the sub-order Homoneura and the super-families Papilionina, Noctuina, and Notodontina respectively.

In this account I have also included a general description of the development and nature of the efferent genital ducts in the above forms. Much confusion prevails on this subject, and I have discussed at length the existing views on it throughout the whole class, supporting my arguments with the conditions which I find in the Lepidoptera mentioned above.

It is with great pleasure that I express my indebtedness to Mr. L. E. S. Eastham, my supervisor of research studies, for suggesting this problem and taking a keen interest in the progress of this research. I am also thankful to Dr. A. D. Imms for various valuable suggestions in preparing the manuscript for publication. To Professor J. Stanley Gardiner I am much indebted for providing me with every facility for work in the Zoological Laboratory, Cambridge.

The material for this work was obtained locally and was partly sent to me from India. In this connexion, I wish to express my sincere thanks to Mr. M. Afzal Husain, Entomologist to the Government of the Punjab, for supplying me with the developmental stages of the silk-worm moth Bombyx mori Linn.

This investigation was conducted during the tenure of a University Scholarship awarded by the Government of the Punjab, India. The grant made by the State is gratefully acknowledged.

MATERIAL AND TECHNIQUE.

The common British forms such as Pieris rapae Linn. and Hepialus lupulinus Linn. were reared in the laboratory. The developmental stages of Earias fabia Stoll. and the silk-worm moth Bombyx mori Linn. were received from the Entomological Section, Department of Agriculture, Punjab, by the courtesy of the authorities.

The specimens were fixed in Bouin's picro-formol and Carnoy's fluids. Real difficulty was experienced in sectioning the last larval and pupal stages of large forms such as Hepialus and Bombyx, on account of the thick outer chitinous cuticle and the amount of air usually retained in the pupae. For this I have devised a modification of Boycott's[1] usual clove-oil celloidin method. It may be here recalled that his method consists in double-embedding of the tissue first in celloidin followed by paraffin, and is suitable only for smaller organisms.

Below I give my modification of his method, which I presume will be found useful for sectioning the bigger forms which are apt to retain large quantities of air in the body cavity.

After proper dehydration and clearing in clove oil the object is kept in each grade of celloidin solution for twenty-four hours, and finally left in the thick solution for a couple of days. This immersion may take a number of days, according to the size of the tissue. It is of great advantage to prick small holes on the pupal covering to ensure the complete penetration of celloidin. After this treatment the material is transferred to a vial containing chloroform, and after the celloidin has set the outer layer of celloidin is removed from around the object. It is at this stage freed from all traces of clove oil by keeping it immersed in chloroform from one to two days, according to the bulk of the specimen. The material is now ready for embedding in paraffin, where it is kept preferably for five to six hours. It is best to be sure now that every trace of chloroform is eliminated from the tissue. This can be easily tested by pouring the melted paraffin over some water in the basin. The presence of any chloroform can be detected by the formation of peculiar crystal-shaped blocks of paraffin. During the embedding of the pupal stages of Lepidoptera it has been found essential to get rid of any air bubbles in the specimen. This is successfully accomplished by keeping it in melted paraffin for about an hour in Hearson's vacuum embedding oven. Having ensured this and the complete penetration of paraffin, the block is made in the usual way and cut into 6–8 μ thick serial sections.

For staining I have used Heidenhain's iron haemotoxylin in all my preparations. Eosin can also be employed as a counterstain in some cases.

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[1] Cited in Bolles Lee's 'Microtomist's Vade-Mecum' (1928).

NOMENCLATURE.

The following is a list of names adopted in this work to denote the different parts of the male genitalia in Lepidoptera.

Uncus (Gosse, 1883).

This forms the dorsal process of the tenth segment. The latter usually remains membranous and retracted below the ninth tergite. The anal tube lies just below the uncus and sometimes projects some distance away from it.

Tegumen (Buchanan White, 1876).

This term is now employed to denote the dorsal part of the ninth segment.

Vinculum (Pierce, 1909).

Saccus in part (B. Baker, 1891).

This is a ventral chitinized band which represents the sternite of the ninth segment.

Valvae (Burmeister, 1832).

These are paired clasping organs which basally articulate with the vinculum and lie in a latero-ventral position.

Gnathos (Pierce, 1909).

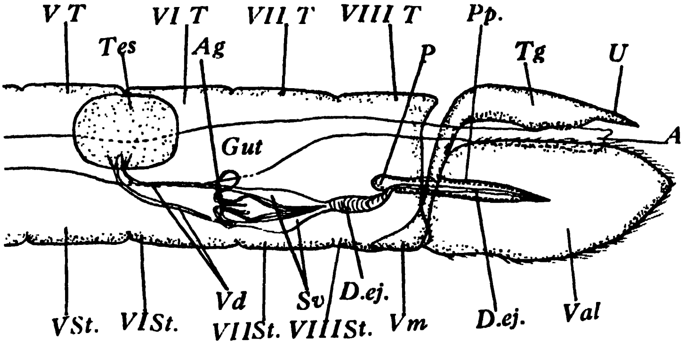
This term indicates a sub-anal structure hinged basally to the uncus, and represents the ventral process of the tenth segment.

Anellus (Pierce, 1909).

It is a cone-like tube or a small, more or less triangular plate which is ventrally supported by the vinculum and on either side by the valvae. It is often extended into lateral process called the 'Anellus lobes'.

DESCRIPTION OF THE GENITAL ORGANS IN THE IMAGO.

Text-fig. 1 illustrates the internal reproductive organs and the external genitalia in Pieris rapae Linn. The testes (*Tes*) are lodged close to the alimentary canal (*Gut*) and just underneath the fifth and sixth abdominal tergites, more or less in a dorso-lateral position. They are fused in the median line and enclosed in a common membrane (Text-fig. 2). From the testes arise a pair of genital ducts, the vasa deferentia (Text-figs. 1–2, *Vd*), which dilate distally to form the vesiculae seminales (*Sv*). The latter are situated on the seventh segment and posteriorly unite with the proximal portion of the ejaculatory duct (*D.ej*). The accessory glands (*Ag*) are a pair of highly convoluted thin tubes which arise from the proximal part of the ejaculatory duct and in close relation with the vesiculae seminales. The ejaculatory duct is formed of two parts as described by Schröder (1912) and Ruckes (1919), the proximal being the 'ductus ejaculatorius duplex' which terminates in a bulbous duct known as the 'ductus ejaculatorius simplex'.



TEXT-FIG. 1.

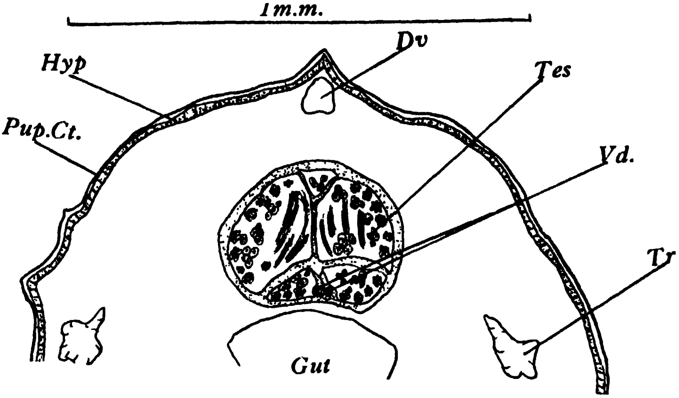
Diagrammatic, showing the internal reproductive organs and the external genitalia in Pieris rapae Linn.

LETTERING FOR ALL TEXT-FIGS.

A, anal tube; *Ag*, accessory gland; *An.l*, anellus lobes; *Ct*, chitinous cuticle; *D.ej*, ductus ejaculatorius; *Dv*, dorsal vessel; *Ej.d'*, ductus ejaculatorius duplex; *Ej.d''*, ductus ejaculatorius simplex; *Gc*, genital cavity; *Gn*, gnathos; *Gut*, intestine; *Hyp*, epidermis (hypodermis); *P*, penis; *Pl*, penis lobe; *Pp*, penis pouch; *Pup.ct*, pupal chitin; *Sv*, vesicula seminales; *Seg*, segment; *St*, sternite; *T*, tergite; *Tes*, testes; *Tg*, tegumen; *Tr*, trachea; *U*, uncus; *Val*, valve; *Vl*, valvae lobe; *Vd*, vas deferens; *Vm*, vinculum; I–X, the different abdominal segments.

The latter enters at the base of the penis and is continued into it.

The external genitalia consist of the modified posterior two segments and their appendages. The ninth tergite or the 'tegumen' (*Tg*) bears a distal process, the 'uncus' (*U*), and the anal tube (*A*) extends below it. The ninth sternum or the 'vinculum' is a chitinous band which bears a pair of lateral outer appendages—the 'valvae' (*Val*). The 'penis' (*P*) is a thick chitinous rod enclosed in an inner membranous layer called the 'penis pouch' (*Pp*) and bears a distinct lumen which is a continuation of the ejaculatory duct (*D.ej*). In Bombyx mori Linn. there arises from the base of the uncus a sub-anal process known as the 'gnathos' (Text-fig. 4, *Gn*). The penis, in this species, is supported by a lateral pair of chitinous sclerites termed the 'anellus lobes' (Text-fig. 10, *An.*1).



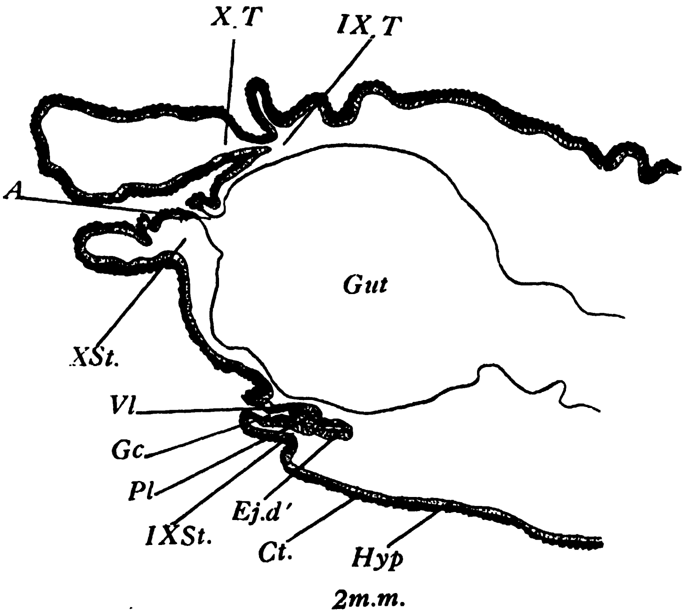
TEXT-FIG. 2.

Transverse section through the sixth abdominal segment on Pieris rapae showing the testes and the vasa deferentia.

DEVELOPMENT.

(*a*) Genitalia.—The following account refers to the development of the genitalia in Pieris rapae Linn. In confirmation of this work, Hepialus lupulinus Linn., Bombyx mori Linn., and Earias fabia Stoll. were thoroughly examined with the same end in view. The principles of development are the same in these insects, therefore reference to forms other than Pieris rapae Linn. has only been made where occasion demanded, as for example in Bombyx mori Linn., which presents morphological features additional to those of Pieris.

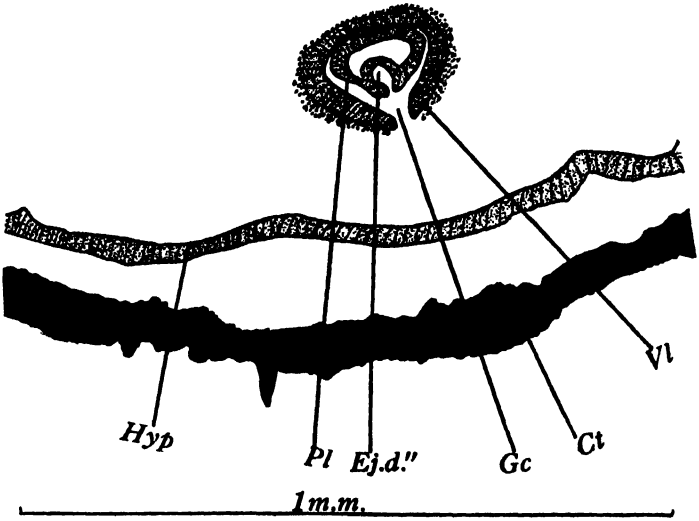
There are ten distinct abdominal segments in the larva. The segments 1–8 bear distinct spiracles and can therefore be easily identified. The ninth is comparatively small and the tenth bears a pair of large pro-legs. The genitalia are formed only towards the end of the larval period, but the genital rudiments become visible soon after the caterpillar has passed through the third moult. At this stage, in a longitudinal vertical section (Text-fig. 3), the relation of different parts is fairly evident. In the earliest larval instar there is an invagination of the body-wall on the



TEXT-FIG. 3.

Longitudinal vertical section through the posterior abdominal segments of the larva of Pieris rapae.

ninth sternite. This becomes wider as the caterpillar passes to the next stage and encloses a distinct pouch or 'genital cavity' (Text-fig. 13, *Gc*). The epidermis begins to thicken at the base of the genital cavity, but the genital rudiments are not distinct till the caterpillar has reached the fourth instar. At this stage (Text-fig. 3) at the base of the genital cavity the epidermal cells form a conical thickening which is later destined to form the penis lobes (*Pl*). The epidermal cells on each side of the genital cavity also become enlarged, but there is no indication of any definite thickening at this stage. Thus it would appear that the rudiments of the penis appear at an earlier stage than those of the valvae. During the end of the larval period the basal thickening of the genital pouch becomes split into a distinct pair of conical lobes—the 'penis lobes' (Text-fig. 4, *Pl*)—which are hung freely in the genital cavity. At this time the epidermal cells bordering the lateral walls of the genital pouch have also become thickened (Text-fig. 4, *Vl*)



TEXT-FIG. 4.

Transverse section through the posterior region of the abdomen in the prepupal stage of Pieris rapae.

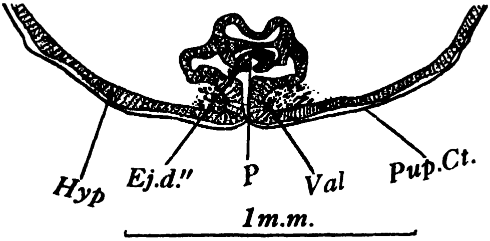
and develop into a pair of lobes, more prominent in Earias fabia Stoll. than in Pieris (Text-fig. 7, *Vl*). They project freely within the genital cavity and represent the 'valvae lobes'. At this stage the surrounding mesodermal cells rich in tracheae migrate into the lumen of these two pairs of cone-like lobes.

Before the larva pupates the penis lobes approximate and fuse in the median line, enclosing a central integumentary depression which forms the 'ductus ejaculatorius simplex' (Text-figs. 5, 6, 8, *E.jd''*). The genital pouch has now almost disappeared by a process of evagination, and the lateral pair of cones lies on the surface close to the ninth sternite and develops into the embryonic valvae (Text-figs. 5, 6, 8, *Val*).

During pupal development the finer modelling of the penis and the valvae takes place. The epidermal cells bordering the valvae (*Val*) show intense activity, so that a layer of chitin is secreted around them. This is particularly evident in the Noctuid Earias fabia Stoll (Text-fig. 8). The penis attains its full size by now, and the 'ductus ejaculatorius simplex' becomes coated with concentric muscle-fibres which give it a peculiar appearance.

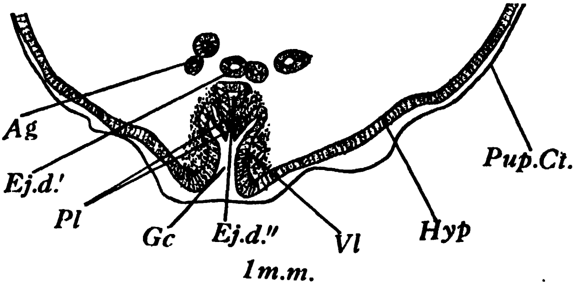
The above mode of the development of the penis and the valvae is identical to that which has been described by Verson and Bisson (1896) in Bombyx mori Linn. But these authors were not in a position to decide whether the fundaments of the penis and the valvae are differentiated from a single broader bud-growth, or that the 'valvae lobes' arise subsequently below the 'penis lobes' and independently of them. Zander (1903) showed in the Pyralid Paraponyx stratiotaria that the former view was more correct, but later workers (Singh-Pruthi, 1924, and others) dissented from it. As I have shown above, the penis lobes are differentiated earlier as compared with rudiments of the valvae from a coniform thickening of the epidermis. It is therefore quite clear that the origin of the 'penis' and the 'valvae' lobes takes place not only independently but also in succession. Besides the Lepidoptera, similar observations have been recorded in Homoptera (Singh-Pruthi, 1924), Orthoptera (Wheeler, 1893), and Hymenoptera (Michaelis, 1900).

The development of the 'uncus' (*U*) and the 'gnathos' (*Gn*) is very interesting. Since these two structures are directly related to the terminal abdominal segments it is desirable to consider at this stage any modifications of the latter during metamorphosis. Since the gnathos is only present in Bombyx of the forms studied the following refers largely to this insect. In the caterpillar there are ten abdominal somites, and segments 3–6 and 10 bear a pair of well-developed pro-legs. The segments 1–8 possess spiracles, and it becomes comparatively easy to mark out the various segments. As pupation proceeds, the ultimate segments of all the forms studied are telescoped within the body, and differentiation into the ninth and tenth becomes less apparent. Externally in the pupa we notice that the



TEXT-FIG. 5.

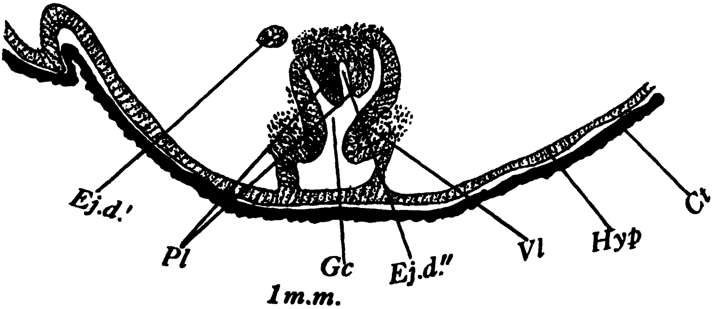
Transverse section through the posterior region of the abdomen in the early pupal stage of Pieris rapae.



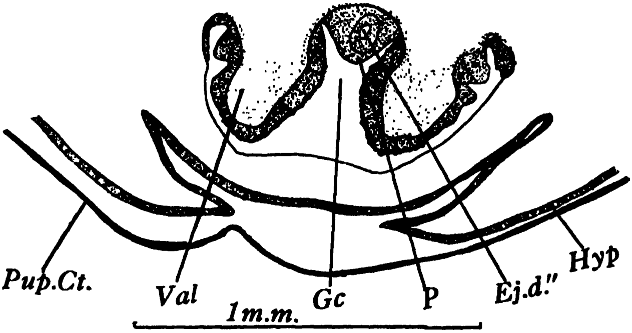
TEXT-FIG. 6.

Horizontal section through the posterior abdominal segments in the early pupal stage of Hepialus lupulinus.

segments 1–7 bear distinct spiracles, while on the eighth they are rudimentary. The ninth and tenth segments closely approximate, and Poulton (1889) showed that 'when a spine is absent and the terminal part of the pupa is rounded, the part above the anus, nevertheless, corresponds to the larval anal flap'. This terminal structure represents a portion of the tenth segment and is known as the 'cremaster'.

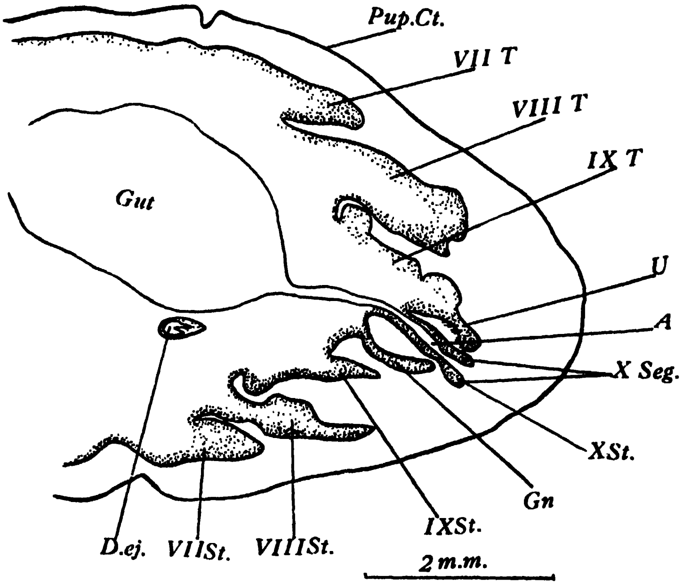


TEXT-FIG. 7.
Transverse section through the ninth abdominal segment in the final instar larva of Earias fabia.



TEXT-FIG. 8.
Transverse section through the ninth abdominal segment of the early pupal stage of Earias fabia.

In an advanced pupal stage, internally the relationship of abdominal segments in the developing imago becomes more evident. A longitudinal vertical section through an advanced pupa of the moth Bombyx mori Linn. shows a congested arrangement of the posterior segments (Text-fig. 9). The seventh to ninth segments are well marked off and possess a distinct tergum and sternum. The ninth tergite (*IX T*), or the tegumen, is distally constricted and joined to a large process, the 'uncus' (*U*), which, in turn, is directly continuous below the tenth segment (*X Seg*). The connexion between the uncus and the tegumen is only secondary, it being actually produced dorsally as a process of the tenth segment. The anal tube (*A*) passes below the ninth tergite (*IX T*) and terminates at the end of the tenth segment (*X Seg*). Ventrally the tenth sternite (*X St*) is produced into a large process, the 'gnathos' (*Gn*). The uncus and the gnathos, therefore, are dorsal and ventral



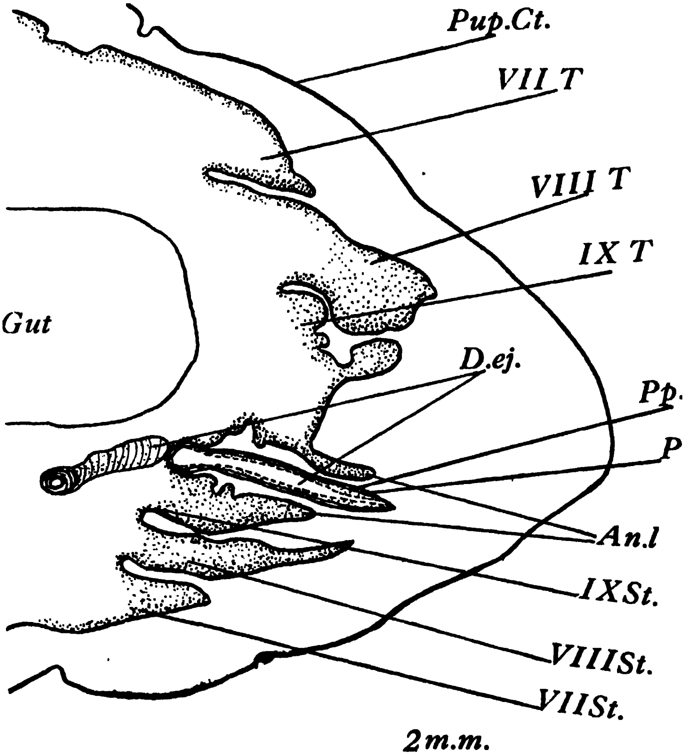
TEXT-FIG. 9.

Longitudinal vertical section through an advanced pupa of Bombyx mori Linn. showing the arrangement of the posterior abdominal segments and their appendages.

processes respectively of the tenth segment. Thus there can be no scepticism regarding the existence of a distinct tenth segment in addition to these two appendages. The limits of the former are well defined, and it is visibly differentiated into a tergal and sternal part (*X St*), and the anal tube (*A*) passes between the two sclerites. In the imago the tenth segment remains membranous and lies more or less in close association with the uncus. It is therefore seldom preserved in preparations of genitalia treated with caustic potash. Probably it is on this account that the uncus and the gnathos ('Scaphium' of Zander and others) are regarded as the tergum and the sternum of the tenth somite (Peytoureau, 1894; Klinkhardt, 1900, and others). Zander (1903) was the first to define the limits of the tenth segment in the pupal stages of the Pyralid Paraponyx stratiotaria, and the above account of Pieris corroborates his conclusions in all essential details.

In the absence of any further ontogenetic evidence confirming Zander's views on these lines, considerable speculation has been indulged in by several morphologists. Busck and Heinrich (1921) believed that the tegumen and the uncus both belong to the tenth somite, the ninth tergite being greatly reduced and continued dorsally as membrane only. In the same way Macgillivray (1923) considered the tegumen as the tenth segment, and regarded the uncus as the distal end of this structure. Differing from these, Forbes (1923) regarded the tegumen as the ninth tergite and the uncus as the 'dorsal part' of the tenth segment. Finally, Philpott (1926), reviewing the uncus in the Micropterygoidea, came to the conclusion 'that the tegumen is formed from the ninth tergite and that the uncus is an outgrowth of that tergite'. I have clearly shown by illustrations from the longitudinal vertical sections through the pupal stage of Bombyx mori Linn. (Text-figs. 9, 10) that the limits of the tegumen or the ninth tergite (*IX T*) and the uncus (*U*) are well defined by the presence of a distinct dorsal constriction. Also, the appendiculate nature of the uncus is evident from its relation with the tenth segment which is withdrawn below it. The tergal and sternal parts of the latter are clearly indicated in my preparations, so that it is easily shown that the uncus and the gnathos are derived as dorsal and ventral processes of the tenth segment.

The ninth sternite (*IX St*) is large and develops a pair of elongate lobiform processes called the 'anellus lobes' (Text-fig. 10, *An.l.*) which lie on either side of the penis (*P*). The intromittent organ or the penis at this stage is fully formed and develops a distinct penis pouch (*Pp*), and its lumen is traversed

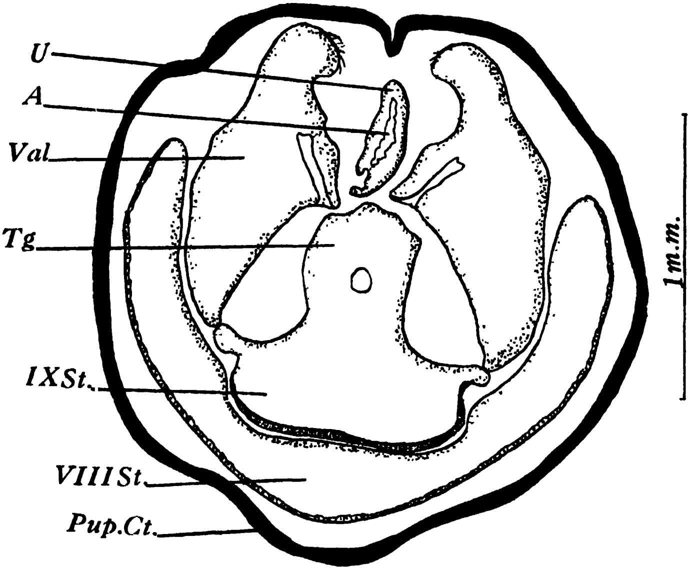


TEXT-FIG. 10.

Longitudinal vertical section through an advanced pupa of Bombyx mori Linn. showing the development of the penis and the anellus lobes.

by the 'ductus ejaculatorius simplex' (*D.ej*) which opens at its orifice.

Towards the end of pupal life the genitalia are presented in their final arrangement. The relation of the different elements which compose the genital armature in Pieris rapae Linn. and Earias fabia Stoll. is fairly evident in a transverse section through an advanced pupa (Text-figs. 11 and 12). Chitinization now proceeds rapidly over the copulatory organs. The valvae (*Val*) assume a broad, leaf-like appearance with a covering of hair and spines. The tegumen (*Tg*) forms a dorsal hood over the anal tube (*A*) which passes below it. The uncus (*U*) has become more prominent and also develops a covering of hairs and spines. The penis is heavily chitinized at this stage and supported on either side by the large anellus lobes (Text-fig. 10, *An.l.*)

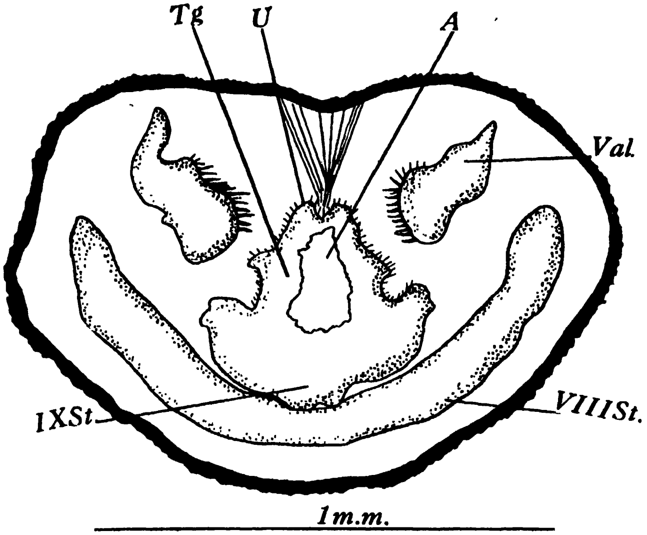


TEXT-FIG. 11.

Transverse section through the posterior abdominal region of the late pupal stage of Pieris rapae.

(*b*) Efferent Genital Ducts.—This account refers precisely to Pieris—the other species confirming it in all essentials.

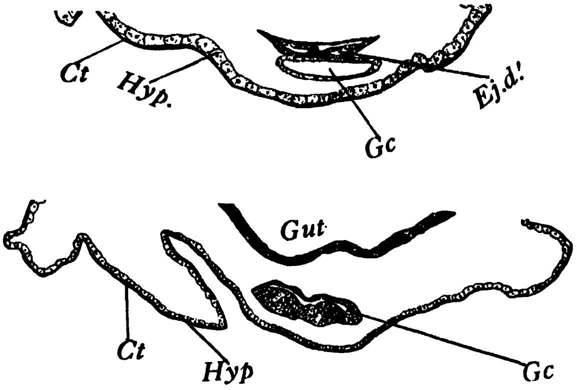
In the caterpillar there persists from the earliest stages a pair of very delicate ducts, the vasa deferentia (*Vd*), which arise from the testes (Text-fig. 4, *Tes*). During larval development they possess a distinct lumen, grow in size, and become convoluted. From the very beginning of larval life each vas deferens terminates on either side of the eighth segment, more or less in a latero-ventral position (Text-fig. 17, *Vd*). In the propupal stage they meet the narrow extensions of the 'ductus ejaculatorius duplex' on the posterior margin of the eighth segment (Text-fig. 18, *E.jd'*).



TEXT-FIG. 12.

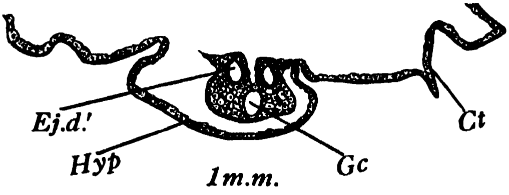
Transverse section through the posterior region of the abdomen in the advanced pupal stage of **Earias fabia.**

In the earliest larval instar of Pieris rapae Linn. the body-wall invaginates to form the genital cavity (*Gc*). At the base of this integumentary depression the epidermal cells differentiate to form a pair of pear-shaped ducts with narrow lateral extensions (Text-fig. 13, *E.jd'*). During the period of larval growth these ducts elongate anteriorily, attain a greater



TEXT-FIGS. 13 AND 14.

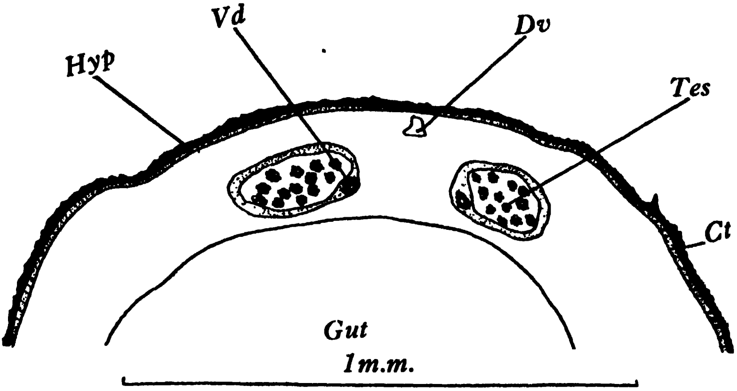
Transverse sections through the eighth abdominal segment (posterior region) of the second larval instar of Pieris rapae.



TEXT-FIG. 15.

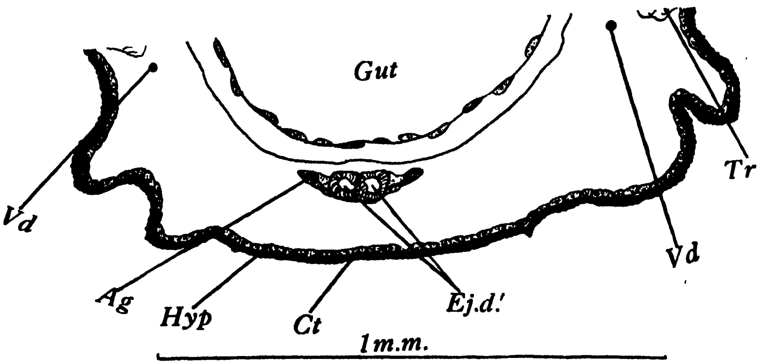
Transverse section through the eighth abdominal segment (posterior region) of the third larval instar of Pieris rapae.

diameter, and become ampulla-like (Text-fig. 15, *E.jd'*). In the fourth larval instar they divide laterally by constriction into two pairs. The dorsal outer pair develops into accessory glands (*Ag*), while the inner fuses to form a single tube, the 'ductus ejaculatorius duplex' (Text-fig. 5, *E.jd'*). Towards the end of larval life the vasa deferentia (*Cd*) and the ductus ejaculatorius duplex (*E.jd'*) approximate and meet below the gut (Text-fig. 18). At first this union is only superficial, but soon after pupation



TEXT-FIG. 16.

Transverse section through the anterior part of the abdomen showing the testes and the vasa deferentia in the fourth instar larva of Pieris rapae.



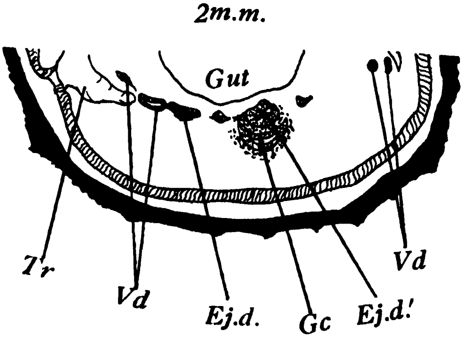
TEXT-FIG. 17.

Transverse section through the eighth abdominal segment in the fourth larval instar of Pieris rapae.

their lumina join. This is remarkably evident in Pieris rapae Linn., Hepialus lupulinus Linn., and Earias fabia Stoll.

During pupation the region of junction between the vas deferens and the proximal end of the 'ductus ejaculatorius duplex' swells up to form the two vesiculae seminales.

In the final larval instar, when the pair of penis lobes is fully differentiated, the intermediate epidermis around them undergoes a secondary invagination. This integumentary depression, the gonopore, deepens and proceeds anteriorly towards and meets the distal end of the 'ductus ejaculatorius duplex' (Text-fig. 6, *E.jd'*, *E.jd''*). When the penis lobes coalesce to form the penis they enclose this tubular invagination (*E.jd''*), which now represents the 'ductus ejaculatorius simplex' of the adult (Text-fig. 8, *E.jd''*). It becomes large and muscular during pupal development.



TEXT-FIG. 18.

Transverse section through the eighth abdominal segment of the prepupal stage of Pieris rapae.

From the histological structure, their nature and position, it is obvious that the vasa deferentia along with the testes are derived from the mesoderm. Their embryonic history, the disposal and growth of the internal epithelium ('Pseudonidi'), is distinctive and cannot be classed with any other tubes in the genital system.

The ejaculatory duct is of purely ectodermal origin. Its proximal portion or the 'ductus ejaculatorius duplex' is usually regarded as of similar derivation to the vasa deferentia (Ruckes, 1919, and others). But in the Lepidoptera I have now shown that this is not the case. In the earliest larval instar this duct is represented by a pair of pear-shaped tubes which are formed by the differentiation of epidermal cells at the base of the genital cavity. As they grow, a distinct internal epithelium is formed, the lumen becomes vesicular, but no chitinous intima is developed. This is partly due to its rather early dissociation and differentiation from the outer epidermis, also because the outer cuticle (*Ct*) is not involved in its formation. Even during larval life their walls are similar to the epidermis which is maintained during the course of development. The 'ductus ejaculatorius simplex' is remarkable in the presence of a distinct chitinous intima. Since this duct is formed at a very advanced stage during larval life, probably it carries with it the chitinous coat on the invaginated epidermal cells, and this is maintained throughout life as the internal chitinous intima. Thus it is clear from the foregoing account that both portions of the ejaculatory duct are ectodermal in origin.

The 'accessory glands' being directly derived from the proximal portion of the ejaculatory duct naturally are of ectodermal origin.

The above account of the origin of the efferent genital ducts in Lepidoptera is almost identical with the account rendered in other orders. Yet there is much confusion in the literature regarding the nature of these ducts, investigators differing more in interpretation than in actual findings. Up till now two main ideas have persisted.

Weismann (1864) was the first to show that the vasa deferentia alone are mesodermal, while the remaining parts of the efferent apparatus are derived from the ectoderm. These conclusions were confirmed by Nussbaum (1884) who, in addition, maintained that the unpaired structures in both sexes arise from the paired rudiments. Palmen (1883, 1884) confirmed these views from his anatomical studies of certain Ephemeroptera and other insects. Quite recently Singh-Pruthi (1924), working on the Jassid Homopteron Idiocerus and the beetle Tenebrio molitor, confirmed Nussbaum's idea that the efferent system in all organs except the vasa deferentia are of ectodermal origin, but he differed from him regarding their actual development, and considered that 'unpaired organs are unpaired from the very beginning, they do not arise by the coalescing of paired organs'.

The second school of thought led by Wheeler (1893) believed that only the common ejaculatory duct is ectodermal, while the vasa deferentia, accessory glands, and the vesiculae seminales are of mesodermal derivation. These conclusions were confirmed by later workers, notably Verson and Bisson (1896) and Ruckes (1919) in Lepidoptera, Christophers (1922) and Christophers and Cragg (1922) in Diptera, and George (1929) in Homoptera and Zygoptera. In their more recent contribution on the nature of such organs in Phlebotomus, Christophers and Barraud (1926) failed to trace the vasa deferentia beyond the eighth segment, and consequently they have become more inclined to Singh-Pruthi's views (1924).

In Lepidoptera, however, I have shown in the preceding pages that the vasa deferentia along with the testes are of mesodermal origin, while the remaining elements in the efferent genital apparatus are derived from the integumental epidermis. In this respect my conclusions are at variance with other workers on this order, such as Verson and Busson (1896) and Ruckes (1919). Perhaps a little discussion on their observations may remove the confusion.

In the earliest larval instar of Bombyx mori Linn., Verson and Bisson (1896) discovered that the vasa deferentia extend up to the base of the genital pouch on the intersegmental fold between the eighth and ninth segments. In this connexion they failed to notice that in Lepidoptera at this stage and perhaps in other insects as well (e.g. Homoptera and Diptera) the vasa deferentia do not extend beyond the eighth somite. This has also been shown by Ruckes (1919) in his anatomical and histological studies of the genital system in certain Lepidoptera. Also, there persists from the earliest stage in the caterpillar a pair of ducts in close association with the genital pouch on the ninth sternum. These tubes divide and coalesce during metamorphosis to form the accessory glands and the proximal part of the ejaculatory duct. They grow during development to meet the ventral extensions of the vasa deferentia on the posterior margin of the eighth abdominal segment. It appears that Verson and Bisson (1896) confused the 'club-ends' of the vasa deferentia described by them with this pair of ectodermal ducts.

Ruckes (1919) following Schröder (1912) believed that the ejaculatory duct in Lepidoptera is histologically differentiated into two regions, the proximal part being the 'ductus ejaculatorius duplex', and the more distal the 'ductus ejaculatorius simplex'. He derived the proximal portion from the mesoderm and regarded the latter as ectodermal in origin. In this connexion he showed that the main difference between these two parts of the ejaculatory duct was the absence in the former of an internal chitinous intima. I have advanced arguments above to elucidate that both portions of the ejaculatory duct are derived from the ectoderm, that is to say, the proximal is formed at a much earlier stage by the differentiation of the hypodermis on the ninth sternum and the distal part grows as an invagination at the same place towards the end of larval life. The development of a chitinous intima undoubtedly is peculiar to the terminal part of the ejaculatory duct, probably owing to its origin as an integumentary depression which later closes up and preserves the layer of outer cuticle thus enclosed. Furthermore, the presence of chitin is not an absolute necessity in an ectodermal organ, as is evidenced by the non-chitinous malpighian tubules of recognized ectodermal nature (Imms, 1929).

In the Cercopid Homopteron Philaenus leucophthalmus L., George (1929) has found that the vasa deferentia terminate on the ninth abdominal sternite from the earliest nymphal stage. He failed to show the existence of a 'pair of ectodermal ejaculatory' ducts lying on the posterior margin of the eighth sternite. It may be here recalled that Singh-Pruthi (1924) referred to the presence of these ducts in the Jassid Homopteron Idiocerus. In this connexion George (1929) has severely criticized Singh-Pruthi's diagram (Fig. 1 A), which shows the vasa deferentia ending at the bases of the 'sub-genital plates' on the ninth segment and the 'paired ejaculatory' ducts extending forward almost to the anterior limits of the eighth segment. Here he fails to see how the 'paired ejaculatory' ducts so situated could 'fuse end to end with the vasa deferentia unless either set of them undergoes a twisting'. Since no such twisting is indicated in any of Singh-Pruthi's diagrams, George was justified in concluding that this fusion, as such, could not be possible. Further, he contends that the 'paired ejaculatory' ducts being more primitive, it would be expected that they would join the vasa deferentia before their connexion is established with the common ejaculatory duct. In this way George (1929) has shown that 'there seems to be little or no evidence regarding the existence of a pair of ectodermal ejaculatory ducts in Homoptera or any other of the higher insects'.

The above arguments advanced by George (1929) are perhaps valid as far as Singh-Pruthi's observations go, since the latter did not explicitly indicate in his drawings what he actually believed. But at the same time there is no other ground on which George could reasonably refute the existence of these ectodermal ducts. I have clearly shown above in Lepidoptera that the vasa deferentia during larval life terminate on the eighth abdominal segment and do not proceed to meet the genital rudiments on the ninth sternum as has been shown by George in Philaenus. In this connexion, my observations corroborate in the main what has been described by Singh-Pruthi in Homoptera (1924) and Christophers and Barraud (1926) in Diptera. Unfortunately, George has omitted reference to Christophers and Barraud's more recent work on Phlebotomus (1926) in which they have stated in unmistakable terms that 'the elements from the testes can be traced at the stage immediately following the last larval ecdysis as far back as well within the eighth segment; but they have never actually at this stage been traced to the rudiments, which is in favour of Singh-Pruthi's contention'. In addition to this valuable piece of evidence, Christophers and Barraud (1926) have also noticed a pair of pear-shaped rudiments (ectodermal ducts) which are present in their usual position as described by Singh-Pruthi (1924) in Homoptera and now by me in Lepidoptera. Their connexion with the vasa deferentia has not been clearly indicated so far by any of these authors, and as Christophers and Barraud (1926) have frankly stated, this aspect of the problem is still open. I have made clear elsewhere in this memoir how exactly the vasa deferentia become linked up with the extensions from these ectodermal ducts towards the end of larval life. The connexion between the proximal and distal portions of the ejaculatory duct is established soon after the former have fused with the vasa deferentia. The whole process is simultaneous, and consequently I do not attach much importance to the last objection raised by George (1929) to Singh-Pruthi's statement that 'the "paired ejaculatory" ducts join the median ejaculatory duct before they join the vasa deferentia'.

The accessory glands being directly derived from the pair of ejaculatory ducts are certainly not of mesodermal origin as has been claimed by George (1929). Their ectodermal nature is evident because they arise by constriction and consequent division of the paired ectodermal ducts, in the late larval instar.

From the foregoing account it is established that in Lepidoptera as in some other insect orders, with the exception of the vasa deferentia, all the elements in the efferent genital apparatus are derived from the ectoderm. The limits of the vasa deferentia are clearly indicated, and evidence is produced to show that its so-called terminal 'ampullae' on the ninth sternite represent the rudiments of the proximal portion of the ejaculatory duct.

SUMMARY.

A general account of the internal reproductive organs and the external genitalia and their development is given.

The 'penis lobes' develop earlier than the 'valvae lobes', and independently of them.

The tegumen is the modified ninth tergite. The tenth segment is visibly distinguished into a tergal and sternal part in the pupal stages, and the anal tube passes between the two sclerites. The uncus and the gnathos are dorsal and ventral processes respectively of the tenth segment. The anellus lobes develop as lateral processes of the ninth sternite on either side of the penis.

The vasa deferentia during larval life do not extend beyond the eighth abdominal segment and lie in a latero-ventral position. They meet the extensions from the ectodermal 'ductus ejaculatorius duplex' during the last larval stadium.

From the earliest caterpillar stage there exists a pair of ectodermal ducts formed by the differentiation of the epidermis on

the ninth sternite. Towards the end of larval life they divide by constriction to form the accessory glands and the 'ductus ejaculatory duplex'. At this stage they extend on either side to meet the vasa deferentia.

The vesiculae seminales develop by distension from the region of junction between the vasa deferentia and the proximal portion of the ejaculatory duct.

The 'ductus ejaculatorius simplex' arises as an ectodermal invagination between the pair of 'penis lobes' during the final larval instar.

It is established that, with the exception of the vasa deferentia, all the remaining elements in the efferent genital system are derived from the ectoderm.

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For explanation of lettering of Text-figures see p. 40.

The Development of Ophiocoma nigra.

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With Plates 5 and 6.

INTRODUCTION.

THE present work, which has occupied my attention for the last two years, was undertaken at the suggestion of Professor E. W. MacBride, F.R.S. A reinvestigation of the development of an Ophiuroid was thought to be necessary, as the only complete work on the subject was done by Professor MacBride himself working on Ophiothrix fragilis as far back as 1907. As he had some doubts with regard to some of his interpretations he asked me to go down to the Marine Biological Laboratory at Plymouth and work on Ophiocoma nigra, which is found in large numbers off the coast of Plymouth.

PREVIOUS HISTORY.

We owe our first knowledge of the development of Ophiuroids to Johannes Müller (1845–1846), who whilst visiting Heligoland happened to observe a larva of an Ophiuroid and named it 'pluteus paradoxus'. In a subsequent paper he described the metamorphosis of this pluteus paradoxus and showed that it was the larva of an Ophiuroid. However, he could not interpret his results fully and failed to identify the coelomic rudiments. In a later paper (1851) he described two further forms of Ophiuroid larvae, one of which he recognized as the larva of Ophiothrix fragilis. In the other, which he called the 'pluteus bimaculatus', he discovered the larval anus of Ophiuroids. Further, he described the rudiment of the water-vascular system as the 'palmate organ', since it possessed five finger-like outgrowths, but was led astray by the five-lobed condition of each primary lobe into imagining that the palmate organ of the earlier larva was identical with the first of these lobes. Müller (1852) also described a worm-like larva of an Ophiuroid. In 1900 Caswell Grave found a similar larva on the American coast and identified it as that of Ophiura brevispina.

The next reference to Ophiuroid development is found in the work of Metschnikoff (1869). Two kinds of plutei were described by him. He was able to obtain only one form with sufficient stages to describe the development in some detail. He discriminated the right and left coelomic rudiments, and the five-lobed hydrocoele which later encircled the oesophagus. He corrected Müller's error about the palmate organs, and showed that, unlike Echinoids, the larval oesophagus persisted in the adult Ophiuroid. He further described the rotation of the madreporic pore to the right side. In this paper Metschnikoff gives valuable information with regard to the viviparous species Amphiura squamata. In Amphiura he described the division of the coeloms into right and left anterior, and right and left posterior coeloms, and the assumption of a five-lobed form by the anterior cavity. He made an important discovery, that the right anterior sac sometimes assumed a rosette-form, and here we find the first definite hint of the paired hydrocoele rudiment.

Further data on the development of Amphiura squamata are provided by Ludwig (1881), Apostolides (1882), Fewkes (1886), Carpenter (1887), Russo (1891), and MacBride (1892). Ludwig's work (1881) referred to the development of the skeleton, and established the homology of the so-called vertebra with a pair of the ambulacral plates of an Asteroid. Apostolides (1882), who worked out the development of this species, put forward the extraordinary view that the endoderm was formed by delamination. Russo (1891) corroborated this statement and added that the coelomic cavities arose as spaces in the mesenchyme. Fewkes (1886) dealt chiefly with the skeleton, but asserted the ectodermal origin of the oesophagus. Carpenter's (1887) work is rather unsatisfactory owing to his attempt to homologize the dorsal plates of Amphiura with the aboral plates of a crinoid.

MacBride (1892) described mainly the post-larval stages and showed that the so-called heart, a problematical organ lying near the stone canal, represented the first rudiment of the genital cells. He discriminated between various spaces confused under the name of the 'axial sinus'. He called the space which he identified later with the right water vascular rudiment 'sinus B'.

The next great advance in our knowledge of Ophiuroid development was made by Bury (1889). He revised the idea of a metameric segmentation of the Echinoderm larvae. In his paper (1889) on the embryology of Echinoderms, he clearly demonstrated the transverse division of the coelom in the larvae of both Echinoids and Ophiuroids. He distinguished between the left anterior coelom and the left hydrocoele. This latter, he believed, arose from the left anterior coelom in Echinoids, but was compelled to assert that it originated from the left posterior coelom in Ophiuroids In a later paper, Bury (1895) gave a complete account of the relationship between the various groups of Echinoderms, and made a valuable suggestion that in Ophiuroids during metamorphosis the left posterior coelom encircled the stomach.

Zeigler (1896) published a short paper on the early development of Ophiothrix fragilis. The only important discovery of his was that the early larva is characterized by a vacuolated crest.

The next important work in this direction was by Grave (1900) on Ophiura brevispina. Because of a cellular plug extending from the apex of the coelom into the archenteric cavity he fancifully concluded that the archenteron was formed by the hollowing out of the inner mass of the larva. He clearly showed that the bi-lobed coelomic vesicle was constricted off the archenteron and later divided into right and left halves. He made an extraordinary discovery: that a second coelomic vesicle was budded from the archenteron and that it subsequently became divided by constriction into two, the anterior half giving rise to the left hydrocoele while the posterior became the left posterior or hypogastric coelom. This notion about the independent origin of the left posterior coelom is unique, and bears a resemblance to the state of affairs in the star-fish

Cribella, the development of which has been worked out by Masterman (1902).

Mortensen (1898 and 1900) made a systematic review of all the varieties of Echinoderm larvae. He was the first to introduce the terms 'Echinopluteus' and 'Ophiopluteus', thus distinguishing the two types of larvae.

The latest important work on the development of Ophiuroids is by MacBride (1907) on Ophiothrix fragilis. He definitely established the origin of the coelom as a single vesicle from the apex of the archenteron, and its later division into three segments on each side. He asserted that the middle right segment, which occasionally assumed a five-lobed form, was the right antimere of the water vascular system. He clearly demonstrated the origin of the hydrocoele as well as the posterior coelom from the anterior coelom. He described the origin of some of the perihaemal spaces and the perioral coelom from the left posterior coelom, which had assumed a ring shape in the course of metamorphosis. He showed that the larval oesophagus persisted, and that the intestine degenerated, and described the origin of the primitive germ-cells from the wall of the left posterior coelom. In short, MacBride's paper gives a complete and satisfactory account of the formation of organs in an Ophiuroid.

MATERIAL AND METHODS.

In the summer of 1930 I went to Plymouth to try to rear Ophiocoma nigra, and obtain all the developmental stages. Ripe males and females were obtained, and the eggs were fertilized. The resulting larvae were introduced into a Browne plunger jar. The larvae were exceedingly vigorous and developed with great rapidity. The question of food for the larvae was the greatest trouble which was encountered. Unlike Echinoplutei and Bipinnariae, it was found the Ophiuroid larvae did not consume the diatom Nitschia to any appreciable extent, although a few diatoms were found in the stomachs of later larvae. Fortunately, in the sea-water contained in the plunger jar there was an unusual amount of phytoplankton, and this probably served as nourishment to the growing larvae, which completed metamorphosis in about forty days. The number of metamorphosing stages of the larvae obtained was rather scanty, but plenty of late larvae were obtained from the plankton towards the end of my stay at Plymouth. The larvae of Ophiocoma nigra were easily distinguishable by the loop at the junction of postero-lateral and post-oral arms. The approximate time for the appearance of various organs are given in the following table:

Free-swimming blastula	24 to 30 hours.
Gastrula	40 to 48 hours.
Formation of coelom	50 to 60 hours.
Stomodaeum meets the gut	About 3 days.
First transverse division of coelom	6 to 8 days.
The appearance of hydrocoele	6 to 8 days.
Left hydrocoele 5-lobed	25 days.
Formation of hydrocoele ring round oesophagus	30 to 35 days.
Degeneration and absorption of larval arms	35 to 40 days.

These times are more or less approximate for artificial cultures. The larvae obtained from plankton were, however, larger and healthier, the rate of development varying with the amount of food present.

The larvae were preserved in osmic acid followed by Müller's fluid and Bouin Duboscq. The latter fixation gave splendid results. A few of different stages of development were preserved in 40 per cent. formaldehyde and transferred to 90 per cent. alcohol for making whole mounts of larvae in order to supplement the observations made on living plutei.

For sectioning purposes the celloidin in paraffin method was used. Most of the sections were cut parallel to the frontal plane of the larva. A few sagittal and transverse sections were also employed when necessary to elucidate special points.

EARLY LARVAL DEVELOPMENT.

Cleavage, Blastulation, and Gastrulation.

The eggs of Ophiocoma are small, being about 0·1 mm. in diameter, and opaque, owing to the presence of yellowish-brown yolk. During fertilization normally a single spermatozoon enters the egg and unites with the egg-nucleus. The membrane of fertilization appears immediately after the entrance of the sperm. Its formation begins at the point where the spermatozoon entered the egg. At this point there is a slight change of shape in the surface of the egg. The membrane completely separates from the egg and stands off at a distance of about half of the diameter of the egg itself.

Cleavage does not occur as rapidly as MacBride describes in Ophiothrix fragilis. Blastula formation occurs only about twelve hours after fertilization. The first cleavage occurs in the vertical plane dividing the egg into two equal parts. Next another vertical division divides it into four cells of approximately equal size. The succeeding cleavages are horizontal, leading to an eight-celled stage, the upper four cells being slightly smaller than the lower four. The succeeding divisions are sub-regular, the cells near the upper pole tending to remain smaller than their fellows at the opposite end.

Cleavage does not culminate in a solid mass of cells or morula, which MacBride found in the artificially fertilized eggs of Ophiothrix fragilis, but as he found in the naturally fertilized eggs of this species, in a thick-walled blastula. Formation of a blastocoele cavity begins as early as the sixteen-celled stage as a space between the upper and lower tiers of cells. As development progresses the blastocoele increases in size. The blastula acquires cilia, and bursts the egg-membrane and becomes free-swimming at the end of about twenty-four hours. Primary mesenchyme cells are budded off from one pole and occupy a large portion of the blastocoele. The opposite pole of the egg-shaped larva is composed of a conical crest of vacuolated cells which probably increase the floating powers of the larva. At the end of about thirty-six hours a slight inpushing, which is the beginning of the archenteron, has appeared at the posterior pole and gastrulation has begun. As gastrulation progresses secondary mesenchyme cells are given off from the apex of the growing archenteron.

Formation of Primary Body-Rods and Coelom.

The slit-like blastopore now assumes a more or less circular form. The larva next assumes a helmet shape owing to the appearance of two lateral outgrowths which are the beginnings of the postero-lateral arms. Most of the mesenchyme cells travel towards these projections, where they go to form the basis of the calcareous rods which support the larval arms. The space between the archenteron and the outer ectoderm is a remnant of the segmentation-cavity or blastocoele, which German authors have termed the primary body-cavity or archiocoele.

The coelom or secondary body-cavity next makes its appearance as a thin-walled sac from the tip of the archenteron. In fig. 9, Pl. 5, it is seen to be in communication with the archenteric cavity. This connexion is cut off and the coelom becomes isolated as a thin-walled vesicle.

Meanwhile, a pit, the stomodaeal pit, lined by columnar cells and having a prominent anterior lip, makes its appearance a little in front of the middle of the ventral surface. By this time the crest has entirely disappeared and the postero-lateral arms have elongated considerably. A transverse ridge, the 'longitudinal ciliated band', has appeared, and is becoming rapidly a horizontal loop. As the postero-lateral arms grow this loop extends into them, thus facilitating the swimming movements of the larva.

The Alimentary Canal.

As the stomodaeum is being formed on the ventral side of the larva, the gut, from which the coelom has detached itself, becomes by constriction divided into oesophagus, stomach, and intestine. The blastopore, the opening of the invagination to form the archenteron, persists as the anus. The stomodaeum now joins the oesophagus, and the alimentary canal of the larva is complete. A thickened tract with masses of cilia projects into the oesophagus. This is the 'adoral ciliated band' and is formed partly by ectoderm and partly by endoderm cells.

Antero-lateral Arms and the Body-Rods.

About the fourth day two projections appear on the anterior pole of the larva. These are the rudiments of the antero-lateral arms and are supported by branches given off from the main skeletal rods which support the postero-lateral arms. At the posterior end of the larva, which is vacuolated, the primary rods branch into the anterior and posterior spikes, which in turn divide into dorsal and ventral branches so that a sort of interlocking arrangement is seen.

Further Development of the Coelom.

We left the development of the coelom at the point where it is constricted off from the apex of the archenteron as a thin-walled vesicle. Having separated off into two portions the coelom travels backwards on either side of the oesophagus to form the right and left coelomic sacs (fig. 10, Pl. 5). Not infrequently at first the right sac is slightly the larger. From the posterior ends of these sacs muscular fibres grow round the oesophagus. These are the 'constrictor muscles' which help the larva in swallowing food. The left coelomic sac at first grows more rapidly than the right one, and it sends out a hollow process dorsally which meets a short ectodermic ingrowth and forms a tube, the 'pore-canal' (fig. 11, Pl. 5). Its external opening is called the 'primary madreporic pore'. At about this time the 'post-oral arms' are formed, as a result of which the ventral surface of the larva becomes concave. The new arms receive extensions from the primary body-rods arising very near the base of those supporting the antero-lateral arms.

Soon after the appearance of the post-oral arms the coelomic sacs undergo a marked change. Now, the left anterior coelom, which has a wider lumen than the right, sends out an extension posteriorly, which is at first solid (fig. 12, Pl. 5). This soon acquires a narrow lumen and separates off from the anterior coelom as the left posterior coelom, and travels backwards so as to be closely apposed to the left side of the stomach. Grave (1900) mentions that the coelom in Ophiura brevis originates as two distinct evaginations from the archenteron, of which the hinder forms the hydrocoele and left posterior coelom, and that the anterior evagination divides into right and left anterior coeloms, the former later giving rise to the right posterior coelom. This mistaken notion is attributable to faulty interpretation, owing probably to stages being missed by Grave, since Ophiura brevis has heavily yolked eggs and consequently undergoes very rapid development. Moreover, it never develops a typical ophiopluteus larva. According to Masterman (1902), in the starfish Cribella oculata somewhat similar results occur, but the posterior evagination gives rise solely to the left posterior or hypogastric coelom, all the other coelomic divisions, including the hydrocoele, being formed from the anterior evagination of the archenteron. This in turn is a mistaken observation as later work on Asteroids has shown. The stage when the left posterior coelom is constricted off from the left anterior coelom must have been missed by Masterman owing to the extraordinary rapid growth of the larva of Cribella oculata.

The right anterior coelom undergoes a similar change to the left and gives rise to the right posterior coelom a little later, about the sixth or seventh day after fertilization. The right anterior and the resultant posterior coeloms are smaller in size than their fellows on the left. Thus, in the case of Ophiocoma nigra, as in Asterias, Echinus, and Ophiothrix, the predominance of the left side of the larva, which plays such an important part in the metamorphosis, over the right is maintained.

External Changes.

No further changes in the larva are noticeable for a few days except the beginnings of the last pair of larval arms, the 'postero-dorsal'. These receive branches from the skeletal rods supporting the antero-lateral arms.

The Hydrocoeles.

At about the sixteenth day after fertilization the posterior end of the left anterior coelom becomes thickened. This is the beginning of the left hydrocoele or the water-vascular rudiment (fig. 13, Pl. 5). This thickening gradually grows in size and becomes hollowed out and is constricted off from the anterior coelom (fig. 14, Pl. 6). However, it does not separate from the latter completely but is connected with it by a narrow neck which is the rudiment of the 'stone-canal'. Bury (1899) supposed that in Ophiuroids, unlike Echinoids, the left hydrocoele originated from the left posterior coelom. He seems to have been led to this erroneous conclusion owing to the close proximity of the left posterior coelom to the hydrocoele. In later stages of development the left posterior coelom almost touches the posterior portion of the hydrocoele, which grows considerably in size, and therefore if an earlier stage is missed one is bound to conclude that the left posterior coelom gave rise to the hydrocoele.

Right Hydrocoele.

The posterior part of the right anterior coelom thickens in the same way as its fellow on the left, a little later—about the twentieth day (fig. 13, Pl. 5). This is the right antimere of the water-vascular rudiment. It corresponds to the right hydrocoele described by MacBride (1903) in Echinus and by Gemmill (1914) in Asterias. MacBride mistook—in Echinus (1903) and Asterina (1896)—another organ, the pericardial sac, which I have described in a previous paper (1932), for the right hydrocoele. This misinterpretation was caused by the fact that the pericardial sac, like the right hydrocoele, arises from the posterior part of the right anterior coelom, the point of origin of the two organs being at different levels. In Ophiothrix, MacBride correctly identified the right hydrocoele, although he found no traces of the pericardial sac in his sections. The right hydrocoele remains as a small thickening with a narrow lumen. It does not constrict off from the right anterior coelom, but its cavity becomes entirely shut off from that of the right anterior coelom though it lies closely apposed to the latter (fig. 17, Pl. 6).

When the larval arms have attained their full length, the left hydrocoele grows anteriorwards, taking on a five-lobed form. The five lobes soon increase in length and become finger-shaped (fig. 18, Pl. 6). The hydrocoele is so close to the wall of the oesophagus that it is very difficult to make out the anterior coelom in living larvae. For the sake of convenience the lobes of the hydrocoele are numbered in figs. 1 to 5, Pl. 5, beginning with the most anterior lobe.

The right hydrocoele does not undergo any such change. Müller (1846) has figured a larva of Ophiothrix in which the right hydrocoele has become five-lobed. MacBride (1907) mentioned that occasionally in Ophiothrix it assumes a five-lobed form, as is shown in his fig. 53. But in Ophiocoma, having cut and examined numerous sections, I have come to the definite conclusion that the right hydrocoele remains throughout larval development as a thickening and does not assume a five-lobed form.

Left Hydrocoele and Stone Canal.

Bury (1899) and MacBride (1907) disagreed with regard to the opening of the stone canal into the left hydrocoele. Bury regards the opening as a fixed point for comparative purposes and, basing his statements on living Ophiuroid larvae, concludes that the stone canal opens into the hydrocoele between lobes 4 and 5. MacBride believed that this opening is situated between lobes 1 and 2. In Ophiocoma (fig. 15, Pl. 6), as in Ophiothrix, the stone canal opens into the hydrocoele between lobes 1 and 2. The external opening of the pore canal is situated opposite the interval between lobes 4 and 5. Since the stone canal is in communication with the inner part of the ring, and the lobes of the hydrocoele project from its outer surface, the point where the stone canal enters the hydrocoele can shift without any difficulty. With regard to the remaining groups of Echinoderms the evidence at present suggests that the opening occurs in a different inter-radius, and therefore further investigation is necessary before any broad conclusions can be drawn.

The Pericardial Vesicle.

At about the thirtieth day after fertilization in serial transverse sections a mass of cells is seen lying near the dorsal surface of the right anterior coelom, connected with the latter by a thin protoplasmic strand. This mass of cells is the rudiment of the pericardial vesicle. This is comparable to the pulsating vesicle of the larvae of Echinus miliaris and Asterias rubens, which arises as a thickening on the dorsal surface of the right anterior coelom, and when constricted off from the latter is connected by a thin strand for a short time. Later it is isolated as a vesicle lying in the mid-dorsal line (fig. 16, Pl. 6). In Ophiocoma this mass of cells soon acquires a cavity and moves over towards the left side, lying more or less in the mid-dorsal line. In Echinus and Asterias larvae I was able (1932) to see this vesicle pulsate. Owing to the extreme opacity of the stomach of the larva of Ophiocoma at about this stage, I was not able to notice any pulsations of the pericardial sac. However, I was able to see pulsations in the just metamorphosed larva of Ophiocoma. Careful focusing through the tissues of the madreporic interradius revealed the presence of a rhythmically pulsating thin-walled cavity. The pulsations are regular and occur once in about twelve seconds. Therefore it is quite reasonable to assume that in the larvae of Ophiuroids the pericardial vesicle pulsates in the same way as in those of Echinoidea and Asteroidea.

Stomach and Intestines.

The intestine is now loose and baggy, and the anus is kept closed except while excreting. The stomach-wall at about this time is quite thick. The cells lining the wall are composed of a layer of rather closely-packed cylindrical cells. The outer ends of these cells are vacuolated. In sections of larvae preserved in osmic acid and Müller's fluid, this vacuolated portion goes black, owing probably to the presence of fat.

The Metamorphosis.

It is rather difficult to fix a definite point which indicates the beginning of metamorphosis in Ophiocoma nigra, since the larvae, as in Echinus and Ophiothrix, remain swimming until the metamorphosis is complete. But in the larvae of Asterina gibbosa and Asterias rubens metamorphosis may be said to begin soon after the larva fixes itself. At fixation the hydrocoele is an open hoop in Asterina. In Echinus the hydrocoele lobes are developed in the form of a hoop but soon become ring-shaped. In Ophiocoma, as in Ophiothrix, the lobes appear while the hydrocoele is still straight, and we may regard the beginning of metamorphosis from the point at which the hydrocoele begins to grow round the oesophagus and assumes a hoop-like form.

The Hydrocoele.

The larva has not been affected in any way with regard to its external symmetry. The arms are quite long and healthy. But changes have been taking place in the hydrocoele. The lobes are long and prominent. The encircling of the oesophagus by the hydrocoele in Ophiurid larvae has already been described by Metschnikoff (1869), Bury (1889), Grave (1900), and MacBride (1907). In Ophiocoma the most anterior lobe of the hydrocoele first grows round the oesophagus over the mid-dorsal line towards the right side, closely followed by the second and third lobes (fig. 7, Pl. 5). The fourth and fifth lobes do not share in the movement as far as I could make out. Now MacBride (1907) mentions that in Ophiothrix the posterior end of the hydrocoele extends over to the right, parallel to the ventral horn of the left posterior coelom. From my observations I am able to make out that the fifth lobe does not completely pass over to the right side over the oesophagus but bends slightly to the right (fig. 8, Pl. 5), to meet the first lobe, which on reaching the right side passes downwards ventrally to the anterior coelom and nearly joins the fifth.

Left Posterior Coelom.

Meanwhile, the left posterior coelom has extended enormously. It has developed two horns which are termed 'dorsal and ventral horns' (fig. 20, *l'.p'.c'.*, *l".p".c".*). The dorsal horn extends forwards along the left side of the larva, outside the hydrocoele lobes, to reach up to about the level of the third lobe. The ventral horn, which is in communication with the dorsal horn at its origin, moves horizontally across the oesophagus to the right side and curves upwards. Eventually at a later stage the two horns fuse, forming a ring outside and parallel to the hydrocoele-ring.

From the inner wall of the left posterior coelom four extensions grow out, alternating between the lobes 2 and 3, 3 and 4, 4 and 5, and 5 and 1 of the hydrocoele (fig. 20, Pl. 6). These outgrowths are the rudiments of the 'Perihaemal' system of cavities. The muscles and the coelomic nerves (Lange's nerves) of the adult Ophiuroid develop later from their walls. These outgrowths are at first thick-walled, and later they acquire a lumen in their middle. At first they are in communication with the left posterior coelom, but are soon entirely cut off and extend as U-shaped canals within the interradii. There still remains the fifth perihaemal diverticulum between lobes 1 and 2 of the hydrocoele to be accounted for. MacBride, in Asterina (1896), as well as Ophiothrix, believed that it originates from the left anterior coelom as an outgrowth. Gemmill (1914), in Asterias rubens, believed it arises from the dorsal horn of the left posterior coelom. From my sections of Ophiocoma it seems to arise from the dorsal horn of the left posterior coelom (fig. 20, Pl. 6), close to the ampulla of the stone canal. The subsequent closure of the perihaemal stalk and the formation of a ring by the horns of the left posterior coelom make it appear as if the diverticulum originated from the left anterior coelom.

By this time the rudiments of the adult arms have appeared. These arise as conical thickenings of the outer wall of the left posterior coelom. Of these outgrowths three are dorsal and two ventral. These thickenings, when first formed, are solid but soon acquire a cavity in them. This cavity is the forerunner of the 'dorsal coelomic canal' of the adult Ophiuroid. A great many of the outer cells of these thickenings go to form the calcareous structures which ensheath the adult. Unfortunately, owing to the methods of preservation adopted, the plates were either dissolved out or destroyed.

External Changes.

Now the first three lobes of the hydrocoele have extended over to the right and downwards so far that the water-vascular ring is almost complete. Soon the external features of the larva are affected. The left side of the oesophageal wall has thickened considerably. The antero-lateral arms have shortened in size and become stumpy. The whole of the anterior pole of the larva is bent over to the right. The madreporic pore and the left antero-lateral arm have inclined to the right half of the body.

The left anterior coelom and the stone canal have shared in the movement and now lie approximately in the mid-dorsal line. The adoral ciliated band has thickened considerably and the thin-walled portion of the oesophagus has been completely pushed over to the right and has diminished in size. It appears as a mere slit encircled by the lobes of the hydrocoele. In short, we may attribute these changes to the tendency of the mouth to move towards the left. Meanwhile, the intestine has become diminished in size and is slanting to the right. Its cells have become vacuolated and are obviously in the first stage of degeneration.

The Primary Tentacles and Epineural Canals.

In the next two or three days the tips of the hydrocoele lobes project into the outer part of the stomadaeum as 'primary tentacles'. Inter-radially outgrowths of ectoderm appear alternating with these tentacles. From these ectodermal ridges flaps grow out on either side. These flaps meet and fuse together to form the 'epineural canals' (fig. 25, Pl. 6) or the closed 'ambulacral grooves' characteristic of Ophiuroids. In this respect the Ophiuroidea resemble Echinoidea, for the epineural ridges of Echinus esculentus arise in a similar manner according to MacBride's (1903) observations.

The next stage in the metamorphosis may be said to have begun with the completion of the hydrocoele-ring by the fusion of its dorsal and ventral ends.

External Changes.

Now the left antero-lateral arm is so far pushed over to the right side that it is in close contact with its fellow on the right (fig. 8, Pl. 5). This is brought about partly by the preponderant growth of the organs on the left side, mainly the encircling of the oesophagus by the hydrocoele, and partly by the shrinkage of the intervening space between the two most anterior arms of the larva. This shrinkage reminds one of the atrophy of the pre-oral lobe in Asterids, especially Asterina gibbosa. Therefore we may venture to suggest that the forepart of the Ophiuroid larva is homologous with the pre-oral lobe of Asterids.

Paired Tentacles and Nerve-Cells.

Now the rudiments of the tentacles begin to appear in the hydrocoele lobes. Each lobe has given rise to three pairs of lateral lobes which are the beginnings of the paired tentacles. The original middle lobe gives rise to the terminal or azygous tentacle on each arm. New tentacles arise by buddings from the main tentacle. The perihaemal rudiments have separated from the left posterior coelom and their cavities have extended in a V-shaped manner, one limb of V extending up each arm. From these extensions, fine lightly-staining wavy fibres with connecting sets of nuclei appear. These are the motor ganglion-cells of the 'coelomic nervous system' (fig. 24, Pl. 6). At the same time other nerve-cells arise from the ectodermal covering of the tentacles. These cells go to form the 'ectodermal nervous system' (fig. 25, Pl. 6) of the adult Ophiocoma. In sections other large cells with few nuclei are seen to arise from the cavity, and similar cellular growths appear from the dorsal coelomic canal. These cells in all probability are the beginnings of the adult 'intervertebral muscles'.

Right Hydrocoele and Pericardial Vesicle.

We must now pause to consider the fate of the right hydrocoele. MacBride (1907) has figured a stage where it has assumed a five-lobed form.[1] Though I have cut a good many sections, I have not been able to get a similar stage. Its normal form in all my sections is as a small thickening of the posterior end of the right anterior coelomic sac. As far as I can make out, the right hydrocoele persists only up to the end of metamorphosis and does not exist in the imago of Ophiocoma. When the hydrocoele forms a complete ring round the oesophagus the right hydrocoele is seen to be a flattened-out structure, and in later stages it thins out and disappears. Therefore, the suggestion that this organ, which serves no useful purpose in the adult mechanism of the Ophiuroid, degenerates as metamorphosis progresses, seems to be quite plausible. MacBride (1907) further states that in the metamorphosing stages of Ophiothrix

[1] **I am satisfied that this figure, which appeared in my paper of 1907, is based on a misinterpretation.—E. W. MacBride.**

fragilis a projection of the inner wall of this organ is noticeable, similar to that which gives it a crescentic form in the larva of Asterina gibbosa (1896). MacBride seems to have mistaken here, as in the case of Echinus and Asterina, the pericardial sac for the persistent right hydrocoele. The inpushing of the ventral wall of this sac goes to form the 'heart' of Ophiuroids. I have already mentioned that the pericardial sac arises in Ophiocoma from the dorsal surface of the right anterior coelom at a higher level than where the right hydrocoele is given off. It arises in a similar way in Echinoids and Asteroids. This sac persists in the adult Ophiocoma as a crescentic vesicle lying beside the stone canal immediately beneath the madreporic plate (fig. 23, Pl. 6). What MacBride calls sinus B in his description and sketches of Amphiura squamata (1892) corresponds to this vesicle. In the just metamorphosed larva of Ophiocoma I have been able to see faint pulsations in this portion. Careful focusing through the madreporic inter-radius, with the help of powerful illumination, revealed faint pulsation in this region. Contractions could be made out with ease from the aboral side. The pulsations were rather regular, occurring once in every ten or twelve seconds. There could be no doubt that this thin-walled crescentic cavity is homologous with the pulsating pericardial vesicle I described in Echinus miliaris and Asterias rubens (1932), and which I regarded as comparable to the pericardium and heart of Balanoglossus. The tissue underneath fills the invaginated heart of the animal.

The right anterior coelom has by now disappeared and the left anterior coelom has enlarged to form the 'ampulla' of the stone canal (fig. 23, Pl. 6). The ampulla is the homologue of the axial sinus of Asterina gibbosa and Echinus miliaris.

The Digestive Canal.

Changes have been taking place in the alimentary canal. The distal portion of the stomodaeum has disappeared owing to its flattening out and its constituent cells being merged in the ectoderm, and the oesophagus is thick walled. This wall is derived chiefly from the left half of the adoral ciliated band. The intestine is reduced to a small solid rod of vacuolated cells. The stomach appears in sections as a solid mass. Later it is seen to send out five projections alternating with the arms (fig. 25, Pl. 6). The adult oesophagus is formed from the inner portion of the larval oesophagus, and the peristomial membrane of the adult Ophiuroid is derived from the outer half which has been flattened horizontally and uncovered owing to the shrinkage of the forepart of the larva. In this respect Ophiuroidea resemble the Holothuroidea, where the buccal membrane and the adult oesophagus are formed in the above manner; but in Echinoids and Asteroids the adult mouth arises as a result of the meeting of ectoderm and endoderm on the left side of the larva.

Final Stage of Metamorphosis.

The last stage in the metamorphosis consists in the disappearance of all larval arms. But the postero-lateral arms persist for a long time, and are the last to be shed. The adult arms have increased in length and have become apposed to the lobes of the hydrocoele by the shrinkage of the ectoderm connecting these two structures. The form of the Ophiuroid, which was pentagonal in the previous stage, is now distinctly five-rayed.

Now, there arises from the left posterior coelom a narrow cavity which insinuates itself between the left posterior coelom and the oesophagus. This is the 'peri-oral coelom' corresponding to that found in Asteroids. This space apparently persists in the adult Ophiuroid as a functionless vestige, but in the Asteriod the dividing wall between the left posterior coelom and the peri-oral coelom breaks down and gives rise to the ten retractor muscles of the stomach. This may be explained by the fact that the stomach of the Ophiuroid is not eversible like that of the Asteroid.

Meanwhile, an interesting change has been taking place in the wall of the left posterior coelom overlying the stone canal. A cluster of darkly-staining long nuclei have made their appearance. These are the 'primitive germ-cells' (*gen.*, fig. 4, Pl. 5). These are at first in the form of a single layer of cells, but later project into the concavity of the pericardial vesicle as solid nodule. This is the rudiment of the 'genital rachis' of MacBride which eventually gives rise to the genital organs by budding.

By this time the oral spines which develop the so-called teeth have already appeared. The ciliated epithelium enveloping the postero-lateral arm degenerates and the animal reaches the bottom and begins to walk on its tube feet. The postero-lateral arms shrink and finally disappear, exposing the spines. The naked spines break off and the young brittle-star emerges.

GENERAL CONCLUSIONS.

We shall now summarize the facts above related and see what conclusions can be drawn from them as to the resemblances and differences in the developmental history of the various groups of Echinoderms.

The development of the coelom forms one of the most important links in connecting up the different groups of Echinoderms. In all cases the coelom arises as a single bi-lobed evagination from the apex of the archenteron. In Antedon rosacea, the only crinoid whose development has been studied, the whole archenteron becomes converted into the coelom which then divides into anterior and posterior portions. The future gut of the larva arises as a pair of outgrowths from the anterior portion. This late appearance of the gut can be accounted for by the fact that it serves no function in the embryo. In the Holothurians the single outgrowth first divides into anterior and posterior outgrowths. The anterior gives rise to the anterior coelom and the left hydrocoele, while the posterior portion divides into right and left halves. In Asteroids, Echinoids, and Ophiuroids, the coelom first divides into right and left halves and these subsequently give rise to the posterior portions and the hydrocoele. In the star-fishes Asterina gibbosa and Cribella oculata the coelomic rudiment does not divide into right and left halves as is the case in Asterias rubens, where the single anterior coelom is formed in the pre-oral lobe by the union of the right and left halves. This peculiarity may be explained by the fact that certain stages are skipped over owing to the shortening of the development in Asterina and

Cribella. Again, in the larva of Ophiocoma, as well as Ophiothrix, the right anterior coelom invariably gives rise to a right hydrocoele. In Asteroids and Echinoids this occurs only as a variation. From the dorsal surface of the right anterior coelom arises a contractile pericardial vesicle in all the three groups. The pericardial vesicle is absent in Holothuroids owing probably to the extreme contractility of the body-wall, and its presence has not been established in a crinoid.

In Echinoids the hydrocoele arises as a disk. Later it becomes ring-shaped. Before the ring shape is complete the hydrocoele has the form of a hoop, recalling its form in Asterias, Ophiothrix, and Ophiocoma. The left posterior coelom gives rise to the dorsal and ventral horns which fuse and form a ring encircling the water vascular ring in Ophiocoma, and a similar thing happens in Asterias. The left posterior coelom in Ophiocoma sends out five pocket-shaped projections. These evaginations are the homologues of the perihaemal spaces of the Asteroid and Echinoid larvae. MacBride mentions that one of these pockets arises from the left anterior coelom in Asterina as well as Ophiothrix; but in Ophiocoma there is no doubt whatever that all the five originate from the left posterior coelom, and this recalls their similar origin in Asterias rubens (Gemmill, 1914).

The atrophy of the forehead of the larva of Ophiocoma is comparable to the degeneration of the pre-oral lobe of the Asteroids. The points in which the development of Ophiocoma differs from that of an Asteroid are: (1) the coelomic cavities are in general smaller and their walls thicker; (2) the fixed stage is absent; and (3) the larval mouth persists in the adult.

In the absence of a fixed stage, and in the consequent retention of the locomotor organs till the tube feet become functional, the larvae of Ophiuroidea resemble the Echinoids. This absence of a fixed stage, which furnishes us with the transitional condition between a bilaterally symmetrical animal swimming freely, and a radially symmetrical animal creeping at the bottom of the sea, may be explained as the result of a tendency to reduce the number of larval stages.

The relatively small size of the cavities and the thickness of the resultant tissues is a general feature of the Ophiuroids.

The Ophiopluteus differs from the Asteroid and Echinoid larvae in the retention of the larval mouth. The Brachiolaria and the Echinopluteus form a new mouth. In Holothuroidea and Crinoidea the mouth persists. The larval stomodaeum of Antedon rosacea does not open into the gut; but still, from its position and structure, corresponds to the stomodaeum of other Echinoderm larvae. When the larva fixes itself the stomodaeum shifts posteriorly and opens out, forming the vestibule from which the tentacles project. In the Holothuroids, according to Metschnikoff's (1869) account, the terminal tentacles when they make their appearance first project into the 'atrium', as the stomodaeum is termed by him. In Ophiothrix and Ophiocoma, owing to the shrinkage of the forehead, the stomodaeum is laid bare and the primary tentacles of the hydrocoele project into it. Now in the Echinopluteus, the tube feet protrude into the amniotic cavity. Therefore the stomodaeum, into which the primary tentacles project in Ophiuroids, is comparable to the amniotic cavity of Echinoids. We may interpret the amniotic cavity as a part of the stomodaeum which is formed separately and then shifted to the left side to occupy the adult position to protect the first-formed tentacles, while the other retains its position in the middle to complete the larval alimentary system.

SUMMARY OF NEW POINTS.

1. Cleavage results in a thick-walled blastula and not a morula as in the artificially fertilized eggs of Ophiothrix.
2. The coelomic divisions are thick-walled and their cavities are much smaller than in Ophiothrix.
3. A right hydrocoele arises as a thickening of the right anterior coelom in all the larvae, but it does not assume a five-lobed form in any case.
4. The right hydrocoele does not persist in the adult, but degenerates as metamorphosis progresses.
5. A pericardial vesicle arises from the dorsal surface of the

right anterior coelom as in Echinoids and Asteroids. It persists in the adult as a thin-walled vesicle lying beside the pore canal.

.6. A heart is formed by the invagination of the ventral wall of this vesicle. Pulsations occur once in about twelve seconds in the ventral wall of the vesicle, in the just metamorphosed forms.

7. During metamorphosis the fifth lobe of the left hydrocoele does not move across the oesophagus to the larval right as in Ophiothrix, but it bends slightly to the right to meet the first lobe which travels towards it after passing round the oesophagus.

8. All the perihaemal spaces arise as pocket-shaped evaginations from the left posterior coelom.

9. The stomach of Ophiocoma is peculiar. The outer ends of the cylindrical cells are vacuolated and stain deep black in osmic acid and Müller's fluid, owing to the presence of fat in them.

10. In post-larval stages the stomach at first appears as a solid mass, but later sends out five projections alternating with the arms.

In conclusion, I have pleasure in expressing my indebtedness to Professor E. W. MacBride, F.R.S., under whose guidance the work was carried out, for his constant advice and constructive criticism which have gone a long way in improving this paper. I must also thank Dr. E. J. Allen, Director of the Marine Biological Laboratory at Plymouth, for providing me with the material required, and giving me facilities to carry out those parts of the work which were done in the Plymouth laboratories. I must also thank my friend Mr. J. K. Deuskar to whose skill I owe the excellent figures in this paper.

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EXPLANATION OF PLATES 5 AND 6.

LETTERING.

al., antero-lateral arm; *b.t.*, buccal tentacle; *cil.ad.*, adoral ciliated band; *cil.bn.*, longitudinal ciliated band; *coe.*, coelomic rudiment; *ep.c.*, epineural canal; *gen.*, primitive germ-cells; *int.*, intestine; *l.a.c.*, left anterior coelom; *l.hy.*, left hydrocoele; *l.p.c.*, left posterior coelom; *l″.p″.c″.*, dorsal horn of the left posterior coelom; *l′.p′.c′.*, ventral horn of the left posterior coelom; *mes.*, mesenchyme; *m.p.*, primary madreporic pore; *mus.f.*, muscle-fibres; *n.c.*, ectodermic nerve-cells; *oes.*, oesophagus; *p.c.*, pore canal; *p.d.*, postero dorsal arm; *ph.*, perihaemal space; *ph. 1 2, 2 3, 3 4, 4 5, 5 1*, perihaemal rudiments originating between lobes 1 and 2, 2 and 3, 3 and 4, 4 and 5, and 5 and 1 of the hydrocoele; *pl.*, postero-lateral arm; *po.*, post-oral arm; *r.a.c.*, right anterior coelom; *r.hy.*, right hydrocoele; *r.p.c.*, right posterior coelom; *sk.*, larval skeletal rod; or so-called teeth of the adult; *st.*, stomach; *st.c.*, stone canal; *stom.*, stomodaeum; *vac.*, vacuolated ectoderm at posterior end of larva; *vac.c.*, vacuolated outer ends of the cylindrical cells of the stomach.

PLATE 5.

Fig. 1.—Optical section of gastrula 36 hours old. The vacuolated crest is well developed.

Fig. 2.—Ventral view of larva 48 hours old.

Fig. 3.—Ventral view of larva 4 to 5 days old. Right and left undivided coelomic vesicles and the calcified rods supporting the arms are shown.

Fig. 4.—Dorsal view of larva about 6 days old. Anterior and posterior halves of the left coelom still connected. Right posterior coelom is also distinguishable.

Fig. 5.—Ventral view of larva about 14 days old. Postero-dorsal arms have just begun to develop and right and left hydrocoeles have appeared.

Fig. 6.—Ventral view of larva, about 20 days old. All the arms have attained their full length. The left hydrocoele has grown forward to about the level of the tip of the left anterior coelom and is five-lobed.

FIG. 7.—Ventral view of larva about 25 days old. Lobes 1, 2, and 3 of the hydrocoele are moving across the oesophagus.

Fig. 8.—Ventral view of larva 30 to 35 days old. Lobes 1, 2, and 3 of the hydrocoele have passed over the oesophagus and travelled downwards so as to be almost in touch with lobe 5 which has slightly inclined towards the left.

Fig. 9.—Longitudinal frontal section of larva 3 days old. Notice the origin of the coelom and the mesoderm cells that go to form the calcareous rods supporting the arms.

Fig. 10.—Longitudinal frontal section of larva about 3½ days old. Right and left anterior coeloms can be made out.

Fig. 11.—Transverse section of larva 4 days old. Ectodermic invagination to form the madreporic pore is shown.

Fig. 12.—Longitudinal frontal section of larva 5 to 6 days old. Outgrowth from the right anterior coelom or the right posterior coelom is still connected with the former. The left posterior coelom has already separated from the left anterior coelom.

Fig. 13.—Longitudinal frontal section of larva 12 to 14 days old. The left hydrocoele is getting constricted off the left anterior coelom while the rudiment of the right hydrocoele has appeared.

PLATE 6.

Fig. 14.—Longitudinal section of larva about the same age as foregoing but slantingly cut. The rudiment of the left and right hydrocoeles are seen.

Fig. 15.—Longitudinal section through a larva about 25 days old showing the opening of the stone canal into the left hydrocoele between lobes 1 and 2 and the opening of the pore-canal to the exterior.

Fig. 16.—Transverse section of larva about 25 days old, showing the left hydrocoele, left anterior coelom, and the pericardial vesicle in their relative positions.

FIG. 17.—Longitudinal frontal section of larva about 30 days old. The

lobes of the left hydrocoele are finger-like. The right hydrocoele is just a thickening at the posterior end of the right anterior coelom.

Fig. 18.—Longitudinal frontal section of larva about the same age as that figured in 17. The lobes of the left hydrocoele are long and prominent. The right hydrocoele is also seen.

Fig. 19.—Longitudinal frontal section of larva about 35 days old. The hydrocoele lobes have encircled the oesophagus. Ventral horn of the left posterior coelom is prominent.

Fig. 20.—Another longitudinal frontal section of the same larva as that shown in fig. 19. The dorsal and ventral horns of the left posterior coelom are clearly marked out. Perihaemal spaces 1, 2; 2, 3; 4, 5 are shown.

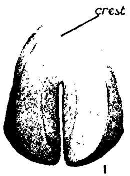
Fig. 21.—Longitudinal section of larva, to same age as the foregoing. Formation of perihaemal space 4, 5 is shown.

Fig. 22.—Longitudinal section of larva rather slantingly cut, showing perihaemal rudiment 2, 3.

Fig. 23.—Transverse section (highly magnified) showing the relative positions of the pore canal, stone canal, ampulla, and the pericardial vesicle in the just metamorphosed larva.

Fig. 24.—Longitudinal section of the just metamorphosed larva. Stone canal, ampulla, pericardial vesicle, primitive germ-cells, &c., are seen.

Fig. 25.—Transverse section of the just metamorphosed larva showing the nerve-cells and muscle-fibres and epineural sacs.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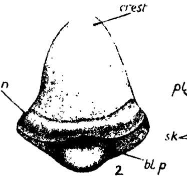
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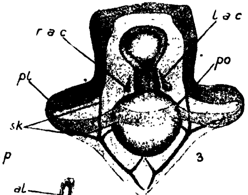
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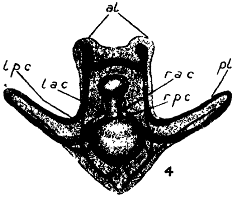
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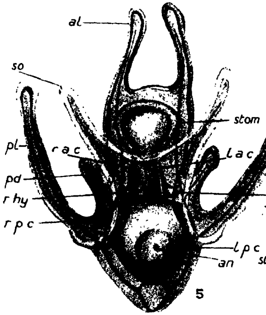
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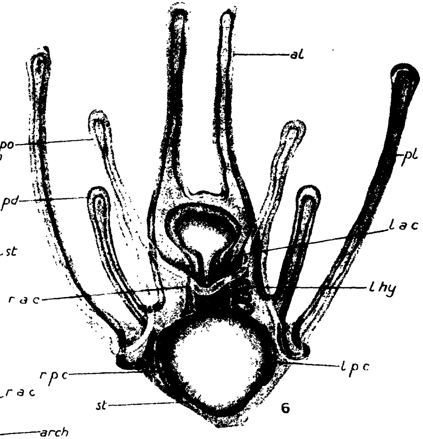
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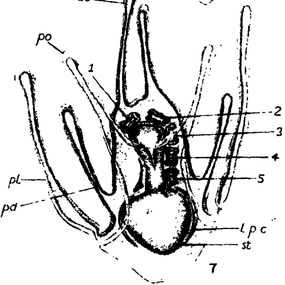
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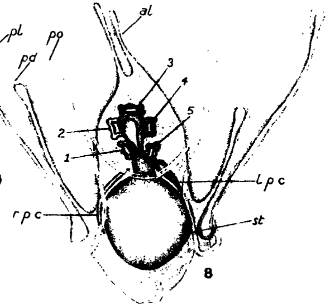
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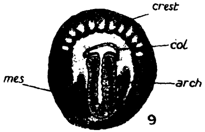
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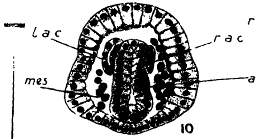
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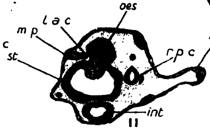
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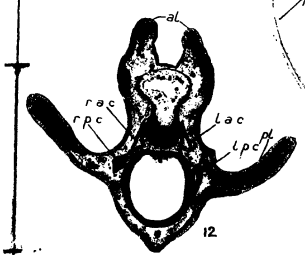
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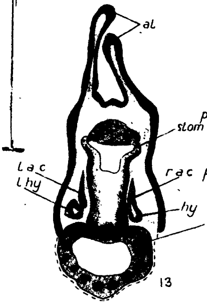
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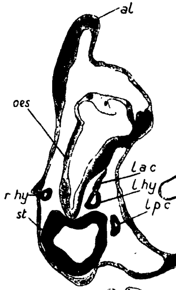

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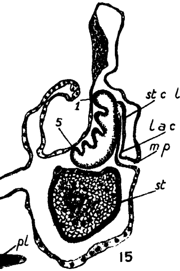

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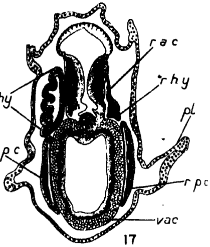

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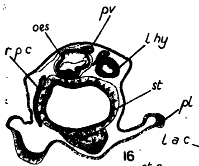

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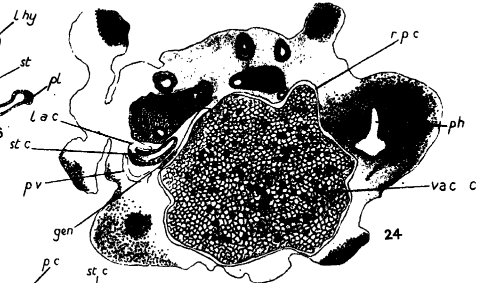

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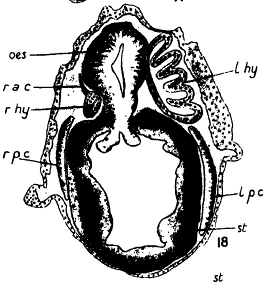

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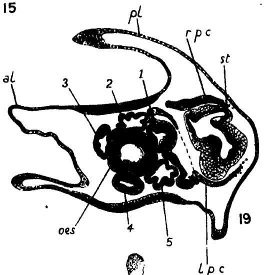

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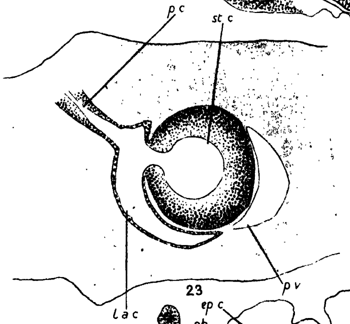

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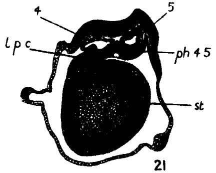

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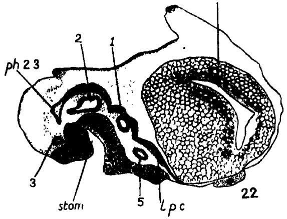

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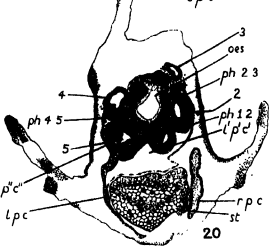

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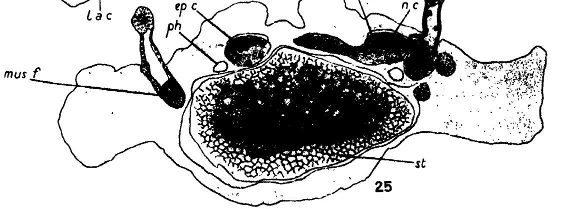

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N. Narasimhamurti, del.

Notes on the Structure and Development of the Female Genital System in Dasyneura leguminicola Lint. (Cecidomyidae-Diptera).

By

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With Plates 7 and 8 and 4 Text-figures.

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I. INTRODUCTION.

THE study of the genital system in insects falls naturally into two sections for discussion, the external genitalia and the efferent system.

In the female, external genitalia of an appendicular nature associated with the eighth and ninth sternites are present in most orders, e.g. Thysanura (Verhoeff, 1902–3, 1910), Orthoptera (Walker, 1919; Nel, 1929, &c.), Hymenoptera (Kraepelin, 1873; Zander, 1899; Seurat, 1899; Haviland, 1921). The homologies of these appendages, their relations to each other and to the segment on which they are located, have an important bearing on the classification of the orders to which the insects belong.

In other orders the appendages may be reduced in number and in size as in the Coleoptera (Verhoeff, 1893; Singh Pruthi, 1924; Metcalfe, 1932), or may be apparently completely absent as in most Diptera (Lowne, 1890–5). This condition is usually correlated with the elongation of the terminal abdominal segments and their modification to form a tubular retractile ovipositor carried within the body when at rest.

In the investigation of any order the questions arise as to whether true appendages are present and, if so, what are their homologies; or whether the ovipositor is derived from the modified terminal abdominal segments.

It is now generally accepted that the efferent system is derived from two sources—the original rudiments which are mesodermal in origin and give rise only to the paired ovaries and a portion of the paired ducts leading from them, and certain secondary structures of ectodermal origin (e.g. Singh Pruthi, George, Nel, Metcalfe). Whether these ectodermal structures are paired in origin as are the mesodermal strands, and the exact extent to which they supplement or replace the latter, are still matters of controversy.

The gonopore in the female is not constantly located posteriorly in a particular segment: in the male, on the contrary, it is always posterior to the ninth segment. In consequence the main efferent ducts in the different orders cannot be considered strictly homologous. This also renders the homologies of the male and female difficult to decide. Again, the relations of the accessory organs—glands, spermathecae, bursa copulatrix—to the main ducts are variable and their exact location has to be considered in each order before their homologies can be determined (Singh Pruthi, George, Nel, Metcalfe).

Owing to this variability it is not surprising that terminology is confusing, and that the same terms are used for structures having the same function but a different origin, in different orders.

For the sake of clearness some attempt has been made to stabilize the terms used in the following paper, in order that any particular duct may at once be referred to its point of origin. It will be found that the terms, with one exception, the vagina, are those which I have already made use of in discussing the development of the female reproductive system in the Coleoptera and the Homoptera.

In the adult the main unpaired efferent duct has been termed the vagina. This opens posteriorly to the ninth sternite and receives dorsally the openings of a pair of accessory glands and a pair of spermathecae proper. The paired ducts leading from the ovaries to the vagina are called the oviducts, no distinction being made between their mesodermal and ectodermal portions.

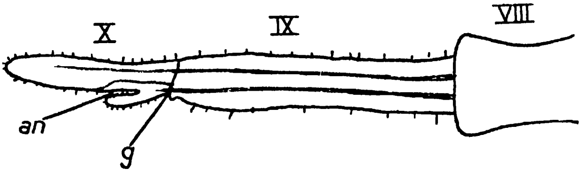
In the pupa the ectodermal invagination arising posteriorly to the eighth sternite is termed the uterine rudiment, its anterior branches the paired or lateral uteri. The ectodermal invagination originating posteriorly to the ninth segment is the spermathecal rudiment, its diverticula the rudiments of the spermatheca proper and the accessory glands. Where it has been found necessary to refer to the invagination posterior to the seventh segment, this is termed the rudiment of the common oviduct. The development of the reproductive system in different families of the Diptera has already been studied in detail (Lowne, 1890–5; Bruël, 1897; Kulagin, 1901; Awati, 1915–16; Christophers, 1922; Christophers and Barraud, 1926; Koch, 1929). In all the cases cited the female gonopore is located posteriorly to the eighth sternite, a curiously primitive position in an order so highly organized in other respects. There are some features in the structure of Culex as described by Christophers, however, which suggest that a duct may be present in some forms which arises posteriorly to the ninth segment. For this reason the Cecidomyidae were chosen for this study as being a family with close affinity to the Culicidae. Larvae, pupae, and adults of Dasyneura leguminicola Lint. being readily obtainable, this species was used as the starting-point for the investigation. The material was fixed in Carnoy's fluid, sectioned, and slides prepared in the usual manner.

It is hoped that an account of the male genital system will shortly be forthcoming, and that the conclusions reached herein will be confirmed by work on other Cecidomyids. The work was carried out at Rothamsted Experimental Station during the tenure of a Fellowship of the University of Wales.

II. ADULT STRUCTURE.

1. The Ovipositor (Text-fig. 1).

Ten abdominal segments are present in the adult female, the last four of which are modified to form a tubular retractile ovipositor. The first six segments are typical body segments of normal size and shape, the seventh is of the same length as the preceding segments, but is much narrower, being about as broad as it is long. The eighth and ninth segments with their intersegmental membranes are greatly elongated and are capable of being withdrawn partially or wholly within the body. Virgin females in the presence of males carry the ovipositor fully extended. After fertilization has taken place the terminal segments are withdrawn into the body and the abdomen then appears to consist of seven segments only. The gonopore is situated at the posterior border of the ninth sternite and is difficult to observe, except when the female is in the act of oviposition when it is seen to be a large and extensible aperture.



TEXT-FIG. 1.

Tip of extended ovipositor. × 18.

LETTERING FOR PLATES 7 AND 8 AND TEXT-FIGS. 1–4.

ag, accessory gland; *an*, anus; *bv* (*sp*), anterior bend in vagina (spermathecal rudiment); *bd* (*sp.* + *ut*), posterior bend in vagina (transition from spermathecal to uterine rudiment); *cc*, chitinous cylinder; *f*, fat body; *g*, gonopore; *lut*, lateral uterus; *mt*, malpighian tube; *mu*, somatic muscle; *od*, oviduct; *ov*, ovary; *rect*, rectum; *rsp*, rudiment of spermatheca proper and accessory gland; *sp*, spermathecal rudiment; *spp*, spermatheca proper; *ut*, uterine rudiment; *v*, vagina; *v* (*sp*), vagina (spermathecal rudiment); *v* (*ut*), vagina (uterine rudiment); II–X, second to tenth abdominal segments.

The tenth segment consists of a long and conspicuous tergite and a small inconspicuous sternite between which the rectum opens. The tergite is much longer than the sternite (fig. 15, Pl. 8).

2. The Efferent System.

The ovaries lie one on either side of the abdominal cavity and extend from the posterior border of the third to the posterior border of the fifth segments. Each is composed of from six to eight ovarioles. The oviducts arise in the fourth segment, and unite ventrally with each other in the posterior region of the fifth segment. The vagina formed by their union is a long and narrow tube which, when the ovipositor is withdrawn into the body cavity, is looped forwards into the second abdominal segment. Here it enters, in company with the rectum, the double-walled cylinder formed by the retraction of the eighth and ninth segments. About half-way along its length the vagina gives rise dorsally to a single pair of tubular spermathecae and a single pair of accessory glands. When the ovipositor is withdrawn the spermathecae lie ventral to the vagina within the double-walled cylinder, while the accessory glands are free within the body cavity.

III. Development.

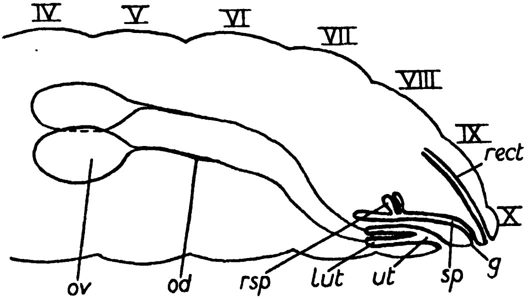
1. The Ovipositor.

Genitalia, in the sense of segmental appendages, being absent, the development of the ovipositor consists of the elongation of the eighth and ninth segments and their retraction into the body cavity. This does not begin until after pupation has taken place. In a young pupa the posterior segments are quite normal in shape and size. Very soon, however, the ectoderm in the ninth segment becomes much thicker (figs. 6–7, Pl. 7), and a groove is formed completely surrounding the anus and rudimentary gonopore. The groove deepens considerably, drawing with it first the whole of the ninth segment (Text-fig. 3) and later the eighth segment also. Eventually a double-walled chitinous cylinder, extending as far as the second abdominal segment in repose, is formed by the retraction of these two segments (Text-fig. 4).

2. The Efferent System.

In the larva the ovaries are present as small rounded bodies, situated in the dorso-lateral region of the sixth abdominal segment. Each ovary is continuous posteriorly with a solid strand of mesodermal tissue which reaches to the posterior border of the segment. This is the whole extent of the efferent system in the larva.

When pupation has taken place the ovaries are seen to have migrated anteriorly into the fourth segment. The oviducts are slender, solid cords of cells extending into the seventh segment (Text-fig. 2).



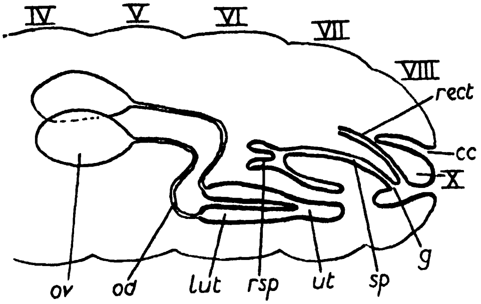
TEXT-FIG. 2.

Diagrammatic longitudinal section through the abdomen of a young pupa.

At the tip of the abdomen the thickening of the ectodermal layer already mentioned takes place. An invagination of this layer arises posteriorly to the ninth sternite and forms the rudiment of the spermatheca. This extends anteriorly into the seventh segment. About half-way along its length, in the eighth segment, it gives off dorsally a pair of blind outgrowths, the rudiments of the spermatheca proper and the accessory glands (figs. 1–7, Pl. 7; Text-fig. 2). Later these become redivided in a horizontal plane, the dorsal pair forming the spermathecae and the ventral pair the accessory glands (figs. 9–12, Pl. 8). About the same time a second invagination arises, posteriorly to the eighth sternite. This is the rudiment of the uterus and is a very short duct almost immediately dividing to form two lateral arms, the paired or lateral uteri. These extend into the seventh segment where they join up with the blind ends of the paired oviducts (figs. 1–4, Pl. 7; Text-fig. 2).

As the pupa matures the ovaries increase in length and extend from the third to the fifth segments. The oviducts acquire a distinct lumen and the point of their union with the paired uteri can no longer be determined.

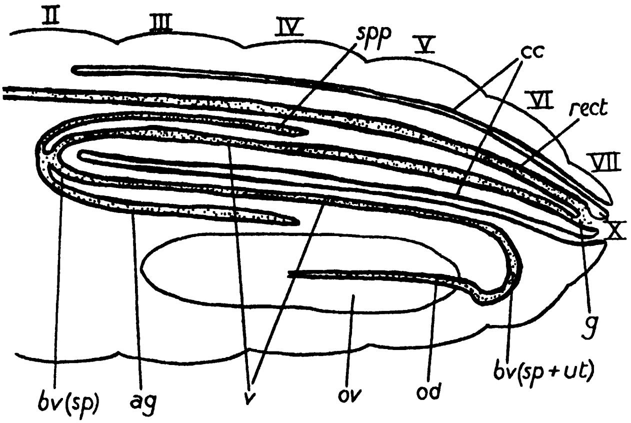
The telescoping of the posterior segments is also taking place, and as this proceeds the rudiments of the spermatheca and uterus are carried forwards as shown in Text-fig. 3. The uterus loses its external opening posterior to the eighth sternite and is moved anteriorly as a whole, the relative positions of its parts being unaltered. The point of division of the paired uteri thus becomes displaced into the sixth segment, while the closed-over posterior end is situated in the seventh segment. The spermathecal rudiment, on the other hand, is thrown forwards in a loop, so that the point of origin of the spermatheca proper and accessory glands is now carried to the anterior border of the seventh segment, while the anterior blind end of the main duct remains in the middle of the seventh segment immediately dorsal to the blind end of the uterine rudiment (see Text-fig. 3). These two blind ends now become opposed to one another, their intervening walls break down, and a through passage is



TEXT-FIG. 3.
Diagrammatic longitudinal section through the abdomen of an older pupa.

established from the uterine rudiment to the spermathecal rudiment: from the ovaries to the gonopore (figs. 13 and 14, Pl. 8). The composite duct formed by the joining up of these rudiments is the vagina.

Growth in the vagina (spermathecal rudiment) now keeps pace with the increasing elongation of the eighth and ninth segments, so that eventually the point of origin of the spermathecae and accessory glands is carried anteriorly into the second segment. The posterior bend (spermathecal + uterine rudiments) in the vagina now lies at the anterior border of the sixth segment (Text-fig. 4).



TEXT-FIG. 4.

Diagrammatic longitudinal section through the abdomen of a fully developed pupa. (N.B.—The proportion of the diameter of the chitinous cylinder to the body cavity has been increased for the sake of clearness. Also, the ovary is shown ventral to the chitinous cylinder instead of dorso-lateral.)

The development of the reproductive system is now complete, and the transit from the pupa into the imago is marked by the shrinkage of the walls of ectodermal origin and their deposition of a chitinous lining.

The vagina is thus formed by the union of two separate ectodermal ducts, the one originating posterior to the ninth, the other to the eighth sternites. The latter loses its external opening, and the functional gonopore is the aperture of the original spermathecal rudiment located posterior to the ninth sternite.

IV. Conclusions.

1. The Ovipositor.

True genital appendages are absent in Dasyneura leguminicola. The posterior abdominal segments are modified in relation to oviposition, as is the case in the female Coleopteron. Kieffer (1900) distinguishes three types of ovipositor in the family Cecidomyidae, each more or less related to the mode of oviposition. These are (*a*) the oviducte à pochette, the type present in D. leguminicola. The ovipositor is usually elongated and capable of retraction. It ends in an appendage which bears the gonopore ventrally, and a tiny median appendage ventral to the gonopore. It is incapable of perforating plant tissues. (*b*) The oviducte aciculaire is also retractile, but is needle-shaped. Its extremity is pointed and the gonopore is situated a little anteriorly to the tip. A tiny ventral appendage is present in some forms and the ovipositor is said to be capable of the perforation of plant tissues. (*c*) The oviducte à lamelles may be retractile, but most often is not. The gonopore is bordered dorsally by a pair of plates, and ventrally one or two plates may also be present. The dorsal plates show diversity of form and may be simple or two or three jointed, fused basally or distinct from one another. Kieffer advances the opinion that the first two types are derived by fusion from the third. From his description the small plates ventral to the gonopore suggest true genital appendages. The small plate in D. leguminicola, however, is dorsal to the gonopore as has already been shown in Mayetiola avenae March. by Ricchello (1929), who regards it as the sternite of the tenth segment. There seems to be some foundation for this statement of Ricchello's, since the anus opens between the two plates.

2. The Efferent System.

The original genital strands, that is to say, the ovaries and mesodermal oviducts, are the only parts of the efferent system derived from the mesoderm. The oviducts in the early pupal stages attain their maximum development and extend into the seventh abdominal segment. Later they are partially replaced by ectodermal outgrowths from the uterine rudiment, so that the oviducts in the adult are partly mesodermal, partly ectodermal in origin. This has already been shown to occur in the Coleoptera (Singh Pruthi, Metcalfe) and Hemiptera (Metcalfe, 1932), though in the latter case the ectodermal portions are derived from the common oviduct and not from the uterus.

The rest of the efferent system is ectodermal in origin. This has already been shown in the Diptera by Lowne, Bruël, Christophers, Christophers and Barraud, and by Koch. It has also been demonstrated in other orders by many workers. A notable exception is George, who finds that the anterior region of the common oviduct in Agrion is derived from the fused mesodermal oviducts: the terminal region is ectodermal.

The main ducts of the efferent system are unpaired in origin. This also is a general conclusion for the Diptera. In the point of origin of the main efferent ducts, however, D. leguminicola differs markedly from other Diptera that have been described.

The gonopore in D. leguminicola is located posteriorly to the ninth sternite. It has a similar situation in Mayetiola avenae March. (Ricchello). According to other workers who have investigated the efferent system in the Diptera, e.g. Lowne and Bruël (Calliphora erythrocephala), Kulagin (Culex and Anopheles), Christophers (Culicidae), Christophers and Barraud (Phlebotomus), Awati (Calypterae), Sturtevant (Acalypterae), 1925, and Koch (Psychoda alternata Say), the female gonopore is situated posteriorly to the eighth sternite. The Cecidomyidae therefore appear to form a distinct group.

It is known, however, that in the females of the Hexapoda generally, the gonopore is not found located constantly posteriorly to a particular segment as in the male, but may lie posteriorly to the seventh (Ephemeroptera—Morgan, 1913; Orthoptera—Wheeler, 1900; Orthoptera (some)—and Dermaptera (some)), to the eighth (Orthoptera—Walker, Nel; Hemiptera—Christophers and Cragg, 1921–2; Singh Pruthi, 1925; George, Metcalfe), or to the ninth segment (Bombyx mori—Verson and Bisson, 1896; Hymenoptera—Kraepelin, Zander, Kulagin, 1897–8; Haviland; Coleoptera—Singh Pruthi, Metcalfe). The gonopore in the different groups cannot therefore be considered strictly homologous, being sometimes the original aperture of the common oviduct, sometimes of the uterus, and sometimes of the spermatheca. The rudiments of all three ducts may be present and their apertures open at some stage in development. Thus in the Hemipteron Philaenus spumarius the common oviduct is present in the early stages. Later the uterus is formed, the common oviduct losing its aperture and opening into the uterus. The latter retains its function as the main efferent duct, and its aperture, posteriorly to the eighth sternite, is the gonopore. An ectodermal duct arising posteriorly to the ninth sternite—the 'spermathecal invagination'—forms the main accessory gland and, retaining its aperture, does not come into communication with the uterus (Metcalfe).

In the Coleoptera, also, all three rudiments are present. The rudiment of the common oviduct, however, has no connexion with the efferent system in the adult, but exists merely in the form of a supporting chitinous rod. The uterus opens to the exterior in the early pupal stages, but later loses this opening and joins up with the spermathecal rudiment. The latter, besides giving rise to the functional spermatheca and accessory glands, forms the main efferent duct; and its aperture, posterior to the ninth segment, is the gonopore (Metcalfe).

In the Macrolepidoptera the ducts arise from the eighth and ninth segments, and although communication is established between them, two separate apertures are retained, the one posterior to the eighth segment being the aperture of the bursa copulatrix and the one posterior to the ninth being that of the 'azygos oviduct' (Jackson, 1888–94).

In the Diptera-Calypterae, as typified by Calliphora erythrocephala, only the uterine rudiment, originating posteriorly to the eighth segment, is present, and gives rise to the main efferent duct and its appendages (Lowne, Bruël).

Similarly the main efferent duct in Psychoda alternata arises posteriorly to the eighth segment; while the accessory gland originates in the same region of the ectoderm, and retains its external opening immediately behind the gonopore. No spermathecae are present (Koch).

In the mosquito Culex the uterus ('common oviduct') and spermatheca proper originate posteriorly to the eighth segment and retain their separate openings. An invagination named the caecus, which receives the opening of the accessory gland, arises posteriorly to the ninth segment; this also retains its aperture and does not come into communication with the uterus (Christophers).

The evolutionary chain in the Diptera suggested above is completed by D. leguminicola. As has been shown, the main efferent duct in this insect is derived from two separate and distinct rudiments, the one originating posteriorly to the eighth segment and the other to the ninth. The former loses its external aperture, and communication is established with the posterior duct which functions as the main efferent channel, its aperture being the gonopore.

With the exception of the fact that the rudiment of the common oviduct is not present in D. leguminicola, its development most nearly approximates to that in the Coleoptera. The table of comparisons on p. 101 clarifies the situation in the Diptera and Coleoptera.

Recently it has been suggested that the line of evolution in the female efferent system in insects lies along the posterior movement of the gonopore (Singh Pruthi, George, Nel, Metcalfe), and this conclusion is further borne out by the evidence from the Diptera given above. An interesting question presents itself here, namely, whether the Cecidomyidae are more advanced in the development of the female reproductive system than are the Calypterae. It is possible that the location of the gonopore posterior to the eighth sternite in the latter group is primitive; but it also may have come about by the degeneration of the spermathecal rudiment, although this process has not been observed hitherto. A further explanation is possible: the number of abdominal segments present in the adult Calypterae is nine. In the male the missing segment is the pregenital

	Diptera.			
Structure.	*Calliphora* (*Lowne, Bruël*).	*Culex* (*Christophers*).	*Dasyneura* (*Metcalfe*).	*Coleoptera* (*Metcalfe*).
Rudiment of common oviduct	—	—	—	Chitinous rod.
Aperture posterior to 7th segment	—	—	—	Closed over.
Rudiment of uterus	Main efferent duct	Main efferent duct	Secondary efferent duct	Secondary efferent duct.
Aperture posterior to 8th segment	Open = gonopore	Open = gonopore	Closed	Closed.
Rudiment of spermatheca	—	'Caecus'	Main efferent duct	Main efferent duct.
Opening posterior to 9th segment	—	Open	Open = gonopore	Open = gonopore.

segment, namely, the eighth (Lowne, Awati). Should the missing segment in the females also prove to be the pregenital, then the gonopore becomes located posteriorly to the ninth segment, and the evolutionary chain in the Diptera suggested above is reversed, with Psychoda and Culex as the primitive forms, Dasyneura as the intermediate phase, and the Calypterae as the most advanced forms.

With regard to the appendages of the efferent system, viz. the accessory glands and the spermathecae: in D. leguminicola these are unpaired and ectodermal in origin, arising from the spermathecal rudiment as in the Coleoptera. According to Nel, the original location of the spermatheca is on the eighth segment. This is borne out to some extent by the work of Christophers, George, and Jackson. Nel regards the position of the spermatheca in the Coleoptera as described by Singh Pruthi as 'exceptional and specialized' (loc. cit., p. 71). This condition is, however, present in D. leguminicola. Furthermore, the spermatheca in Culex as described by Christophers arises in exactly the same position as the accessory glands in Psychoda alternata as described by Koch. In the latter insect spermathecae are absent. If it be supposed that the spermatheca in Culex is really the accessory gland while the caecus is the functional spermatheca, then the comparison between the Cecidomyidae and the Culicidae is carried a step farther.

There seems to be no reason why the spermathecal function, as well as the gonopore, should not undergo a posterior migration.

Jackson's view that the accessory glands are paired in origin has been recently revived to some extent by Christophers and Barraud, George and Nel. In all cases the glands described by these authors arise directly from the ectoderm. In the Coleoptera and in D. leguminicola the glands arise as paired outgrowths from an originally unpaired structure and must therefore be considered as fundamentally of unpaired nature.

It is unfortunately impossible at the present time to compare the structure of the male with that of the female, but it is hoped that this will appear in a later paper.

V. SUMMARY.

1. Genitalia of an appendicular nature are absent in D. leguminicola, the terminal abdominal segments being modified to form a tubular retractile ovipositor.
2. Apart from the ovaries and a portion of the paired oviducts, the efferent system is unpaired and ectodermal in origin.
3. The gonopore is posterior to the ninth sternite, and is derived from the primitive spermathecal invagination as in the Coleoptera.
4. The uterine rudiment is present during the early stages of development, but later becomes closed over, discharging its contents into the duct derived from the spermathecal rudiment.

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EXPLANATION OF PLATES 7 AND 8.

LETTTERING FOR PLATES 7 AND 8 AND TEXT-FIGS. 1–4.

ag, accessory gland; *an*, anus; *bv* (*sp*), anterior bend in vagina (spermathecal rudiment); *bd* (*sp.*+*ut*), posterior bend in vagina (transition from spermathecal to uterine rudiment); *cc*, chitinous cylinder; *f*, fat body; *g*. gonopore; *lut*, lateral uterus; *mt*, malpighian tube; *mu*, somatic muscle; *od*, oviduct; *ov*, ovary; *rect*, rectum; *rsp*, rudiment of spermatheca proper and accessory gland; *sp*, spermathecal rudiment; *spp*, spermatheca proper; *ut*, uterine rudiment; *v*, vagina; *v* (*sp*), vagina (spermathecal rudiment); *v* (*ut*), vagina (uterine rudiment); II–X, second to tenth abdominal segments.

PLATE 7.

Figs. 1–7.—Transverse sections through the posterior abdominal segments of a young pupa from anterior to posterior.

Fig. 1.—The spermathecal rudiments and paired uteri. ×173.

Fig. 2.—The spermathecal and uterine rudiments. ×173.

Fig. 3.—Origin of uterine rudiment. ×187.

Fig. 4.—Section a little posterior to fig. 3. ×180.

Fig. 5.—Origin of the common rudiments of the spermatheca proper and accessory glands. ×217.

Fig. 6.—The telescoping of the posterior segments. ×140.

Fig. 7.—The gonopore. ×167.

Fig. 8.—Transverse section through the fourth abdominal segment of an older pupa showing ovaries. ×103.

PLATE 8.

Figs. 9–15.—Transverse sections through the abdomen of an older pupa from anterior to posterior.

Fig. 9.—Showing anterior bend in the vagina. ×217.

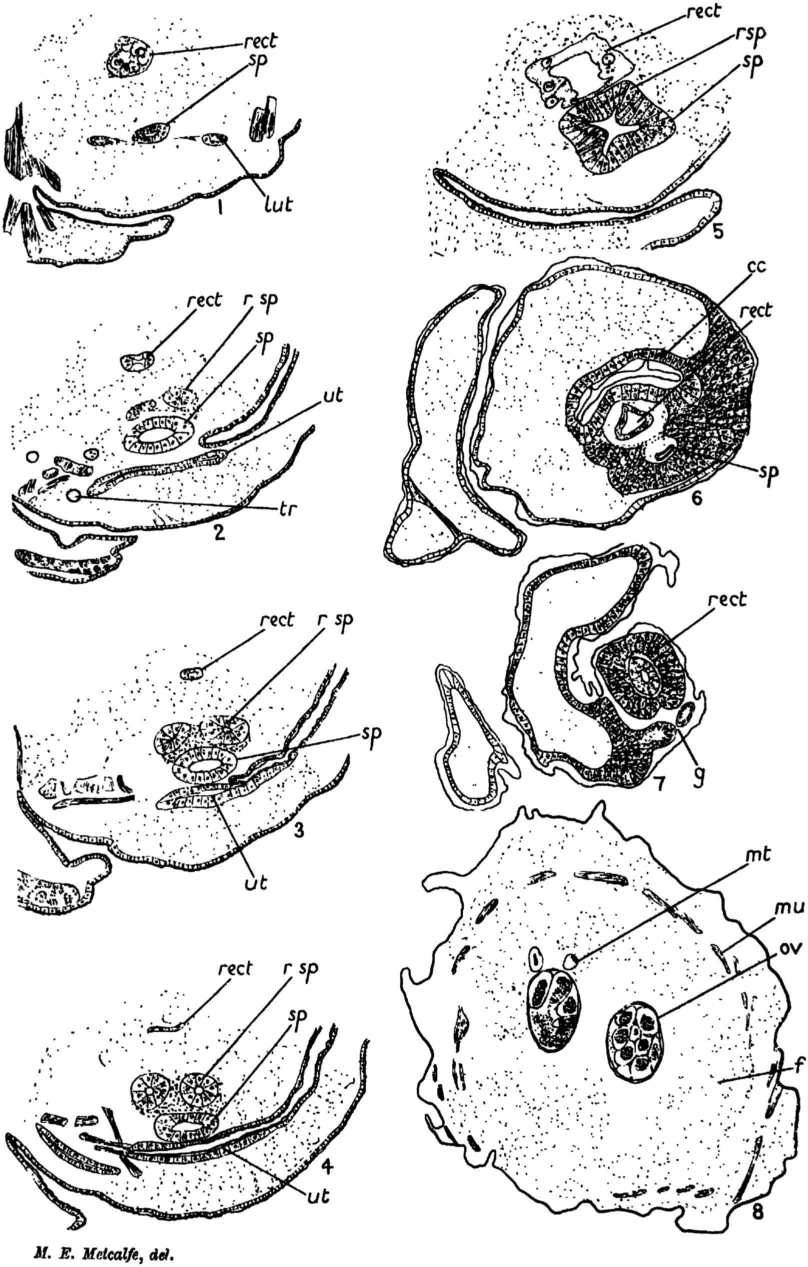
Fig. 10.—Separation of two limbs of vagina. ×233.

Fig. 11.—The telescoped posterior segments and blind end of uterus. ×253.

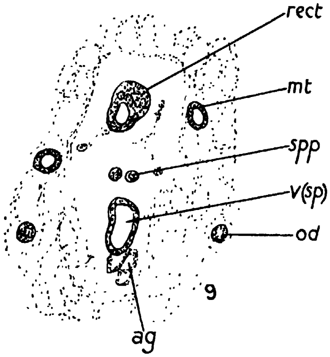
Fig. 12.—Division of the uterus. ×267.

Figs. 13 and 14.—The posterior bend and union of the uterus and spermathecal rudiments. ×233.

Fig. 15.—The tenth segment posterior to the anus. ×233.



M. E. Metcalfe, del.



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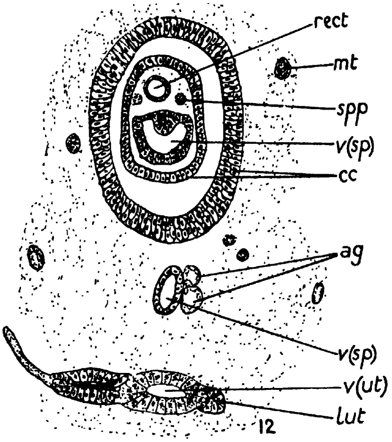

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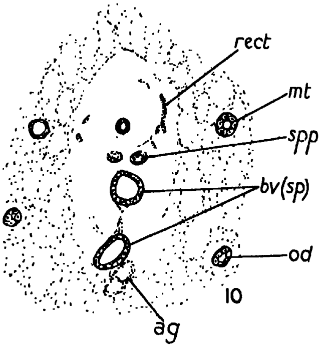

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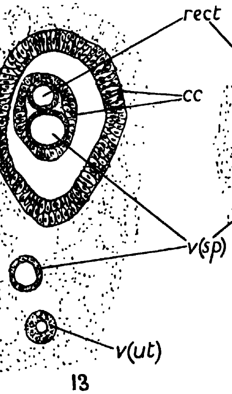

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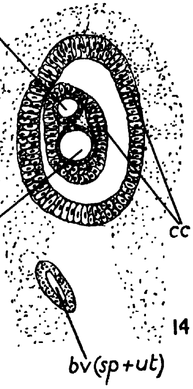

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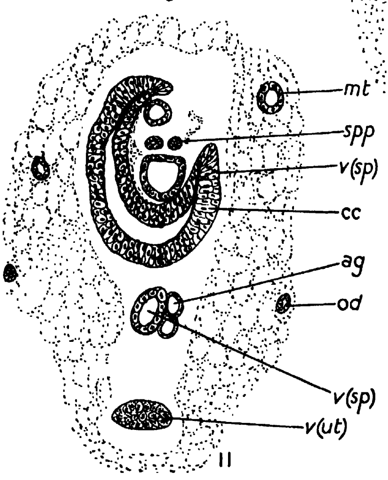

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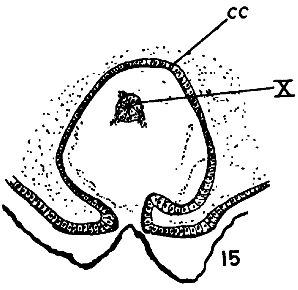

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M. E. Metcalfe, del.

On the Occurrence of 'Hepato-pancreatic' Glands in the Indian Earthworms of the Genus Eutyphoeus Mich.

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With Plates 9 and 10.

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1. INTRODUCTORY AND HISTORICAL.

IN 1883 Beddard (1) instituted the genus Typhoeus on the basis of an earthworm from Calcutta and recorded the presence of a series of about six pairs of intestinal glands lying on the dorsal surface of the gut of this earthworm which he named Typhoeus orientalis. In 1889 he (2) described another species of this genus from Darjeeling as T. gammiei, and gave a brief description of the structure of these glands in the following words: 'The alimentary canal presents only one other feature of interest, and that is the presence of intestinal glands already recorded in T. orientalis. The glands are, however, not confined to this genus, since they exist in much greater numbers in Megascolex, and have also been described by Horst in Acanthodrilus, and by myself in Eudrilus. In Typhoeus the glands agree in their minute structure with those of Megascolex, but differ anatomically in the fact that the two glands of each pair become fused together on the middle dorsal line of the intestine, and also in the fact that the glands of consecutive segments are connected. The minute structure bears a very close resemblance to that of the calciferous glands.'[1] Beddard does not describe the histology or blood-supply of the glands, but gives a diagram of a transverse section of a portion of one of the glands which is reproduced in fig. 6, Pl. 10.

In 1895, in his monograph on the Oligochaeta (**3**), Beddard says that in all the three species of Typhoeus (T. orientalis, T. gammiei, and T. masoni), of whose anatomy we possess at all sufficient data, the intestine is furnished with a series of about six pairs of reniform glands lying on its dorsal surface. He adds that the glands are made up of a much-folded membrane, their interior being thus divided up into numerous compartments by the folds which seem to anastomose, and that the structure of the glands is extremely like that of calciferous glands. Further, in his monograph Beddard (**3**, p. 64) mentions these glands as occurring only in Megascolex and Typhoeus and says that they are unknown otherwise in the Oligochaeta. He drops out both Acanthodrilus and Eudrilus from the category of worms possessing 'intestinal glands', presumably because the descriptions of the glands in these two cases are very meagre and have not since been confirmed.

Since 1895 several other species of Eutyphoeus (the new generic name for Typhoeus) have been described; Stephenson (**10**, 1930), in his recent admirable memoir on the Oligochaeta, records as many as twenty-six species of this genus, all from India, especially the Gangetic Plain. In systematic work it is not necessary to open the middle of the worm, since all characters of systematic importance can be made out by a dissection of the anterior portion of the worm alone; but in spite of this fact, these 'intestinal glands' on the dorsal surface of the intestine have been described in several species, and Stephenson (**9**, 1923) considers 'that these glands may not improbably be a general character of the genus, though they have not, as a rule, been noted by recent observers, who have not usually opened the intestinal regions of these worms'.

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[1] The spaced words are mine.

Like Beddard, Stephenson (**10**, 1930) also records these glands only in Megascolex coeruleus and Eutyphoeus. The only description available so far of these glands is that given by Beddard (**2**) which has been quoted above, his diagram being reproduced in fig. 6, Pl. 10. It will be seen, therefore, that these glands have not been properly investigated, and that their significance has not been realized. Beddard worked only on preserved specimens, and was really concerned mainly with characters of systematic importance rather than with the minute structure and function of the glands.

In 1923 G. S. Thapar (Proc. Tenth Ind. Sci. Congress) communicated a paper, the published summary of which reads as follows: 'In certain species of Eutyphoeus are found a few paired glands in connection with the alimentary canal. They occur behind the middle of the body, covering the intestine from above in a few consecutive segments. The author, after describing the histology of these glands and the condition of the intestine in this region, proceeds to compare these glands with other glands in connection with the alimentary canal of earthworms. A brief discussion on the probable origin of these glands in Eutyphoeus is also given.'

While engaged in working out the morphology and physiology of these glands, I was struck by their extraordinary resemblance to the so-called 'liver' or 'hepato-pancreas' of other Invertebrates. Not only do they resemble the 'liver' in their position, structure and development, but their blood-supply, the digestive secretion they discharge into the gut, and the presence of glycogen granules in the cells corroborate their hepato-pancreatic character.

The chloragogen cells of Oligochaeta have been credited with a nutritive function by Schneider (**7**, 1896), and Liebmann

(5, 1926) has recently revived the view that the chloragosomes are storehouses of nutriment; but the existence of a definite localized 'liver or hepato-pancreas' is so far unknown amongst the Oligochaeta, or even amongst the Annelida, although one would expect that this important organ or its representative must be present in animals at the Annelidan grade of organization.

Owing to other pressing engagements and also to the fact that these worms are available only during the monsoon months (July to September), the work has taken a long time, but a number of observations and results have already been communicated to the Indian Science Congress (1930) and to the United Provinces Academy of Sciences (December 21, 1931). In carrying out the physiological part of the work I have received considerable help from my friend and colleague Dr. S. M. Sane of the Chemistry Department at Lucknow, and my best thanks are due to him. Dr. J. G. Mukerjee of the King George's Medical College has also been very helpful, and I have pleasure in recording my thanks to him. I am also thankful to Mr. M. L. Bhatia for his help in the illustrations.

As the paper is being published jointly, it will be appropriate to state that the senior author is responsible for the observations and conclusions, as also for the preparation of the manuscript and the diagrams. The junior author rendered valuable help in the experimental part of the work by preparing the material and generally assisting in the work. The first person singular in the text refers to the senior author.

2. THE 'HEPATO-PANCREATIC' GLANDS OF EUTYPHOEUS.

(*a*) The Position of the Glands.

All previous observers mention that these glands occur in about five or six successive segments in the middle of the body, but they have not recorded their exact segmental position. I have carefully counted the segments of Eutyphoeus waltoni Mich. and find that the total number of segments varies from 190 to 210 and that the glands always occupy five segments—seventy-ninth to eighty-third, both inclusive—(fig. 1, Pl. 9). If we take into account, therefore, only the segmental position of the glands, they would seem to lie quite in front of the middle of the body, but this is actually not the case. The glands are really situated behind the middle of the body, the discrepancy between their segmental position and their actual site being due to the fact that the anterior segments of the worm are much larger in size as compared with the posterior segments. In fact, the anterior half of the worm is made up only of 65 to 70 large segments, while the posterior half contains as many as 135 to 140 small segments. Thus it is that the glands, although they occur in segments 79 to 83, actually lie behind the middle of the body.

One other fact with regard to the position of these glands, which has not been observed by previous workers, may be recorded here: that the glands always occupy the last five typhlosolar segments of the intestine. On opening the gut of Eutyphoeus we find that the typhlosole is very feebly developed, as for example in Pheretima, and forms a very low and narrow ridge which projects for a very short distance into the lumen of the gut (fig. 2, Pl. 9). It is merely a fold of the intestinal epithelium and corresponds to Stephenson's (**10,** 1930) first type of typhlosole characteristic of the families Megascolecidae and Glossoscolecidae, in which only the 'blood of the gut-sinus fills out the space within the epithelial fold, containing also a quantity of loose connective tissue'. This type of typhlosole is to be distinguished from the second type, i.e. that of the Lumbricidae and Microchaetinae in which all the coats of the intestine are folded in to form the typhlosole, forming a prominent ridge which projects a good distance into the lumen of the intestine. Again, the typhlosole in Eutyphoeus, as in other earthworms, does not extend along the whole length of the intestine; it stops short of the eighty-fourth segment—so that we can distinguish an interior typhlosolar region of the intestine extending up to and including the eighty-third segment and a post-typhlosolar region[1] occupying the last 107 to 127 segments. The intestinal glands always occupy the last five typhlosolar segments of the

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[1] Beddard has suggested the term 'rectum' for this post-typhlosolar region of the intestine.

intestine. As seen in fig. 2, Pl. 9, the typhlosole at its posterior end (segments 79 to 83) no longer remains a low and narrow ridge, but becomes very prominent and forms a thick and flat swollen mid-dorsal ingrowth, this appearance being due to the presence of 'intestinal glands' overlying and depressing the typhlosole in this region.

The 'hepato-pancreatic glands', therefore, lie within and above the epithelial fold of the typhlosole (figs. 2, 3, and 4, Pl. 9) at the posterior end of the typhlosolar region of the intestine, there being no trace of a typhlosole behind the region of the glands. In other words, the typhlosole in its last five segments carries the 'hepato-pancreatic glands' on its dorsal surface.

(*b*) The Structure of the Glands.

In a live worm the glands are easily recognized through the translucent skin as a very vascular red patch behind the middle of the body. When a large number of different kinds of live worms are brought to the laboratory, I have invariably sorted out Eutyphoeus from other worms by recognizing its vascular intestinal glands. On opening a freshly narcotized specimen in normal saline, the glands are seen as prominent, paired, rounded swellings on the dorsal surface of the gut lying on either side of and beneath the dorsal vessel. Each pair of glands is again bilobed through a shallow indentation about the middle of its length. The glands exhibit rhythmic contractions, the wave of contraction passing from their outer edges to the mid-dorsal line. Since the glands are richly permeated with blood-capillaries, the blood also follows the rhythmic wave of contraction, and one can easily observe under a binocular dissecting-microscope the flow of blood in the capillary network from the outer edges of the glands towards the dorsal vessel.

In the seventy-ninth segment the glands are usually small, but they go on increasing in size in the succeeding segments, the best-developed glands being the last pair in the eighty-third segment. In serial sections of a worm the glands of the seventy-ninth segment measured $616\mu \times 366\mu$, while those of the eighty-third segment measured $1350\mu \times 833\mu$. In fact, the last pair of glands are not only the largest in size, but they also bulge backwards and overlap the intestine, so that in transverse sections of this region the posterior bulgings of the glands appear as paired rounded structures lying separately above the intestine. We may note here that although five distinct pairs of glands are visible (fig. 1, Pl. 9) on a superficial examination, actually all of them form one continuous structure; since, as Beddard observed (**2**, 1889), not only are the pair of glands in each segment fused together in the mid-dorsal line, but even the glands of successive segments are continuous with one another. In fact, we should speak of all the five pairs of glands as one large hepato-pancreatic gland extending over five consecutive segments.

The glands (figs. 3 and 4, Pl. 9) are formed of a large number of branching lamellae and lobules of varying sizes and lengths massed together. Just as the epithelial folds of the typhlosole enclose between them part of the blood of the gut-sinus, similarly each fold or lamella of the intestinal gland encloses a blood-sinus in its double fold of glandular epithelium. The spaces between the adjoining lamellae are well-defined and form the channels into which the secretions of the gland-cells are discharged. Generally, the ventral part of the gland nearest the gut shows simple or branched lamellae hanging freely in the cavity of the gland, but the dorsal and lateral parts of the gland are much more solid and consist of interlacing and anastomosing lamellae forming distinct lobules which in sections present a honeycombed appearance, each cell of the honeycomb being a lobule surrounded by a blood-sinus and enclosing a gland-ductule within its layer of glandular epithelium (fig. 5, Pl. 10). In sections passing through the greater part of the glands of the eighty-third segment, one can see the whole gland filled up with tubular lobules, each lobule being formed of a single layer of glandular epithelium surrounding a gland-ductule, and separated from the adjoining lobules by sinusoid blood-capillaries. The free lamellae hanging in the cavity of the gland are altogether absent in the posterior region of the glands. The three essential elements of a gland-structure, i.e. the secretory epithelial cells, a blood-supply, and a basement membrane holding the secretory cells in position, are all present. Each of the five glands is covered on the outside by the muscular layers of the intestine and the outer visceral peritoneal layer, while ventrally it is lined by the ciliated intestinal epithelium forming the roof of the gut. The muscular layers are very thin and lie only on the surface, but do not extend into the substance of the gland. Further, the cells of the visceral peritoneum are also very small and flat, unlike the tall swollen cells of the other parts of the gut.

The glandular cells are more or less rhomboidal—even polygonal—in outline, and are arranged in single layers along a well-marked basement membrane which encloses a blood-sinus. Some of the cells have a straight free margin, but in most cells the free margin presents a rounded swollen appearance. The nucleus lies either in the centre of the cell or near its free margin; it is more or less rounded and shows within it distinct darkly staining spots. On an average, each cell is about 11 μ in height and about 14 μ in width, while the nucleus is about 6 μ in diameter. The cells bear neither cilia nor rodlets on their free surfaces, the cilia or rodlets being confined to the cells of the intestinal epithelium only. We may note in passing that while the whole of the intestinal epithelium of the gut in front of the glands and in the region of the glands themselves bears cilia or rodlets, the epithelium of the gut behind the glands, i.e. the folded epithelium of the rectum, does not bear any cilia or rodlets (fig. 8, Pl. 10).

The glandular cells are no doubt secretory, their free surface being the secretory surface. One cannot fail to notice a collection of secretory granules within the outer bulging surface of the cells as well as in the cavities of the glands. The shape and structure of the cells remind one strongly of hepatic cells. A trained observer, like my friend Capt. J. G. Mukerjee of the Medical College to whom I showed my sections, remarked that the cells were exactly like the human liver-cells. The secretions from the gland-cells are discharged into the ductules and cavities of the glands, whence they are poured into the intestine usually through two paired openings (fig. 3, Pl. 9) in each of the five glandular segments.

These openings of the glands into the intestine are distinct and well-defined; in a series of sections through the five glands the number of openings into the intestine was found to be as follows:

(1) Seventy-ninth segment—one median unpaired opening.
(2) Eightieth segment—one median and two paired openings.
(3) Eighty-first segment—two paired openings.
(4) Eighty-second segment—two paired openings.
(5) Eighty-third segment—two paired openings.

Thus there are altogether eighteen openings of the glands into the intestinal lumen, some median (fig. 5, Pl. 10) but generally paired (fig. 3, Pl. 9). Each opening is elongated and slit-like, 30 μ to 44 μ in length and 11 μ to 17 μ in width as measured from serial sections. The ciliated intestinal epithelium extends upwards into these paired openings, so that the actual openings are lined with the intestinal epithelium (fig. 5, Pl. 10) and not with the glandular epithelium, the latter being confined to the actual substance of the glands.

(c) The Blood-supply of the Glands suggesting a Hepatic Portal System.

As we have already mentioned, the distinguishing character of the intestinal glands in a living specimen is their blood-red appearance which makes them easily visible through the translucent body-wall of the worm. The minute sinusoid capillaries are thickly interwoven to form a close network so that the glands are richly permeated throughout with blood (fig. 2, Pl. 9; figs. 5 and 7, Pl. 10). That a copious supply of blood courses through the glands is easily observed by opening a freshly narcotized worm and examining the exposed glands under a binocular dissecting microscope. The glands are seen contracting rhythmically and their blood-capillaries alternately filled with and emptied of blood. The blood is seen to enter the glands along their outer edges, run through the capillaries of the glandular tissue, and to come out again to pass into the longitudinal dorsal vessel through two pairs of intestino-dorsal vessels in each of the five glandular segments. As the glands contract and relax, a wave of blood is seen passing from the outer edges to the middle line of the glands during the passage of the blood to the median dorsal vessel. As many as twenty-one to twenty-five such waves per minute were counted in freshly narcotized worms.

All along the length of the post-typhlosolar intestine (from the eighty-third segment to the posterior end of the worm), i.e. in the region of the gut behind the intestinal glands, a prominent blood-vessel—the ventral intestinal sinus—runs along the mid-ventral line of the intestine (fig. 2, Pl. 9). In transverse sections (fig. 8, Pl. 10) this sinus is a very prominent structure, and is seen bulging out from the floor of the intestine into the intestinal lumen. In size it is as big or even bigger than the dorsal vessel and is always seen full of blood in preserved specimens.

The direction of blood-flow in this sinus is from behind forwards, as has been verified by me by cutting the sinus at various places and observing from which of the cut ends the blood flows; it always flows from the posterior cut end. In the eighty-third segment this ventral-intestinal sinus forks into two (fig. 2, Pl. 9), and each of the two branches at once runs dorsalwards and continues along the outer edges of the glands, emptying all its contained blood into them. These lateral glandular sinuses, therefore, take the place of the ventral-intestinal sinus in the region of the intestinal glands and form prominent blood-channels lying at the outer edges of the glands (figs. 2 and 4, Pls. 9 and 10). In the region of the glands these lateral glandular sinuses are in communication not only with an enveloping sinus, which goes all round the periphery of the glands between the thin outer muscular layers and the glands proper, but also with the general capillary network of the glands. The enveloping sinus is also continuous with the extensive network of sinuses permeating the lamellae and lobules of the glands. If we bear in mind the large size and extent of the ventral intestinal vessel (fig. 8, Pl. 10) and the large amount of blood contained in it, we cannot help concluding that the greater part of the blood from the intestine in the last 107 to 127 segments passes into the ventral-intestinal sinus. It flows forwards in this sinus, and is carried through the two lateral glandular sinuses into the sinusoid capillaries of the glands. The blood courses through these capillaries of the glands and passes into the dorsal vessel through the two pairs of very short intestino-dorsal vessels in each segment.

The size and disposition of the intestino-dorsal vessels in the different regions of the body of Eutyphoeus is interesting. In the region of the body in front of the glands two pairs of ring-like intestino-dorsals are very prominent in each segment: they run all round the gut and are seen bulging on its outer surface (figs. 1 and 2, Pl. 9). As they are all along in communication with the general intestinal sinus they, no doubt, carry the blood from the intestine into the dorsal vessel. Behind the region of the glands, however, they are very short blood-vessels and do not go round the gut (fig. 2), as they do in the anterior region. They emerge from the dorsal surface of the intestine and immediately enter into the dorsal vessel; in short, the intestino-dorsals are very much reduced in size and extent in the post-typhlosolar region of the intestine. They no doubt receive a small quantity of blood from the gut and convey it to the dorsal vessel. But the greater part of the blood of the intestine of this region really goes into the ventral-intestinal sinus, which is very large and prominent—even larger than the dorsal vessel—and discharges its contained blood into the intestinal glands. In the region of the glands itself the intestino-dorsals are again very short, as the dorsal vessel comes to be very closely connected with the glands—it is almost imbedded between the glands of the two sides in each of the five segments, as shown in fig. 5, pl. 10. In fact, there can be no doubt that the blood from the glands directly enters the dorsal vessel. Not only is it actually seen doing so in a freshly narcotized worm, but that this is the direction of flow is confirmed by the disposition of the valves (fig. 5).

We can thus describe the blood-supply of the glands by saying that the two lateral glandular sinuses break up into a network of sinusoid capillaries within the substance of the gland; these capillaries join together again and form two pairs of intestino-dorsal vessels in each segment to convey the blood from the glands into the dorsal vessel.

We thus get a semblance of a portal system in connexion with these hepato-pancreatic glands: the blood from the intestine is collected into the ventral-intestinal sinus; from there it goes to the glands, in which it breaks into a close network of capillaries; these capillaries join together in each gland to form two pairs of intestino-dorsal vessels which discharge their contained blood into the contractile dorsal vessel in each of the five glandular segments.

(*d*) The Development of the Glands.

Interesting light is thrown on the nature of the glands by a study of their development. They arise as median dorsal outgrowths of the endodermal epithelium of the intestine in the seventy-ninth to eighty-third segments. In an embryo, about 15 mm. in length, the glands are seen to extend over five segments, but the development is more rapid in the posterior segments than in the anterior ones. The endodermal epithelium of the gut in these early embryos consists of very tall cells full of very small albumen granules which do not take up the stains, the nuclei alone being stained red with borax-carmine. In the anterior segments the mid-dorsal wall of the gut is seen to give out a hollow outgrowth; the tall albumen-laden cells of the intestinal epithelium grow upwards, become gradually smaller and smaller in size until they pass into the gland-cells which are absolutely free from albumen-granules and are stained deeply with borax-carmine (fig. 9, Pl. 10). The glands themselves at this stage consist of rounded or elliptic follicles formed of a single-layered epithelium, each follicle enclosing a small or large cavity which will later on form, no doubt, one of the many ductules of the adult gland. At the regions of these glandular outgrowths one can easily make out a long narrow canal through which the lumen of the gut communicates with the lumen of the gland. The cilia or rodlets which form a characteristic feature of the gut-epithelium of this region in the adult worm are completely absent from the gut-epithelium of the embryos.

In the middle and posterior regions of the glands the mid dorsal region of the gut changes its character. Its cells are no longer large and full of albumen granules, but have undergone a proliferation into smaller cells, more or less free of albumen granules. These differentiated cells take up a deep borax-carmine stain and are seen to bud off gland-cells from their dorsal surface (fig. 10, Pl. 10). The intestinal cells and the gland-cells are very closely associated in this—the posterior—region of the glands. We may note here that these differentiated cells are confined only to the mid-dorsal region of the gut; the cells of the remaining parts of the gut are still large and full of albumen granules. Further, on the dorsal surface of the glands the peritoneal cells, which are also filled with albumen granules, much larger in size than those present in the endodermal epithelial cells, also bud off cells which migrate into the substance of the glands and make their way in between the follicles of the gland-cells. These cells, originating from the mesodermal peritoneal cells, go to form the sinusoid capillaries of the glands.

We may conclude, therefore, that the glands arise as dorsal hollow outgrowths from the roof of the intestine and are reinforced in the posterior region by the budding off of cells from the mid-dorsal region of the gut. Later, cells are budded off from the peritoneal epithelium of the gut, make their way into the endodermal glandular tissue, and lead to the development of sinusoid blood-capillaries. The substance of the gland is therefore entirely endodermal, while the blood-capillaries are mesodermal in origin.

3. The Physiology of the Glands.

The most important question about these glands is their function. Bearing in mind Beddard's suggestive observation that 'the minute structure of these alimentary glands bears a very close resemblance to that of calciferous glands', the first step was to compare these glands with the 'calciferous glands'. Fortunately, Eutyphoeus itself possesses definitive calciferous glands, a single pair in segment 12, and these form a constant character of all the species of this genus, just as the 'intestinal or hepato-pancreatic glands' form another constant character. We get, therefore, both the intestinal glands and the calciferous glands in one and the same earthworm. The calciferous glands have been described in detail by Stephenson and

Prashad (8, 1919), and a comparison between the two kinds of glands as regards their nature is interesting.

The very first consideration in the comparison of the glands is their respective position. One salient feature which marks off at once the 'intestinal glands' from the 'calciferous glands' of all earthworms is that while the latter are always situated in the oesophageal region, the former are situated just about the middle region of the intestine, where the greater part of the food of the earthworm would be digested and absorbed. Besides this essential difference in their respective positions, there are differences in structure between the two types of glands. There is no doubt that both kinds of glands are formed essentially of folds of the gut-epithelium, oesophageal epithelium in the one case and the intestinal epithelium in the other; but this does not indicate identity of nature or function, since we must bear in mind the fact that in other animals the gut-epithelium is known to be capable of giving rise to structures entirely different in nature and function. The origin of lungs, liver, and pancreas from the gut-epithelium of different regions of the body of a vertebrate is a case in point. In comparing, however, the structure of the calciferous and intestinal glands in an adult Eutyphoeus we must note: firstly, that the intestinal glands are much more solid structures than the calciferous glands. While the latter consist of a large number of simple vertical lamellae only sometimes united by synapticula, the former consist of lamellae which interlace and anastomose so freely with one another as to produce an almost solid honeycombed structure. Both in transverse and longitudinal sections (figs. 3 and 11) the intestinal glands present greater complexity and a higher degree of differentiation than is seen in the calciferous glands. Again, there are other differences. Stephenson and Prashad (8, 1919) record the presence of cilia or rodlets on the free borders of the cells of the calciferous glands; these cilia or rodlets are entirely absent from the cells of the intestinal glands, being confined to the definitive gut-epithelium only. Further, the sinusoid blood-capillaries of the intestinal glands are enclosed by a distinct basement-membrane, while in the case of the calciferous glands a basement-membrane, according to Stephenson and Prashad, is often not to be made out in well-fixed preparations, though well seen in badly fixed specimens.

That the intestinal glands are not functionally 'calciferous' is easily proved by the fact that they never secrete calcium carbonate. While the calciferous glands in segment 12 always show copious white calcareous particles of milky lime under a binocular microscope, the intestinal glands never show any traces of lime, nor could they be shown to contain any trace of calcium even on chemical examination.

Experiment 1.—The intestinal glands from twenty specimens of Eutyphoeus were ignited on a platinum foil, and the foil, together with the ashes, was placed in dilute hydrochloric acid in a test-tube. The acid was boiled and the solution of the ash was transferred through a filter-paper into another test-tube. Ammonia was added until the solution was alkaline. On the addition of ammonium oxalate no precipitate of calcium oxalate was formed, showing the absence of calcium.

The same test was applied to the definitive calciferous glands of the twelfth segment, when whitish calcium carbonate ash could be detected immediately on ignition. This was confirmed by boiling the ash in dilute hydrochloric acid and testing with ammonia and ammonium oxalate, when a white precipitate of calcium oxalate was formed, showing the presence of calcium.

This test definitely negatived the possibility of the intestinal glands being functionally calciferous in nature.

Whatever may be the real function of the calciferous glands in segment 12, it seems highly improbable that glands identical in nature to them would occur again in the same earthworm sixty-seven segments behind.

Having satisfied myself by observation and experiment that the intestinal glands are not calciferous in function, the next step was to determine the nature of their secretion. The fact that the glands open into the gut generally by two pairs of minute apertures in each of the five segments—eighteen openings in all, to be exact—strongly indicates that the secretion of the glands is discharged into the gut through these apertures.

The possibility of the glands directly absorbing nutriment from the gut is almost completely excluded, since the openings are very minute and the glands are fairly well segregated from the gut—it is practically impossible for the contents of the gut to come in contact with the surface of the cells of the gland.

Attempts were made, therefore, to determine the nature of their secretions. A preliminary test was made with the extract of the gland (strength 2·5 per cent.) to determine its action on calcified milk.

Experiment 2.—Five cubic centimetres of the gland-extract was added to 5 c.cm. of calcified milk and kept at a temperature of 40° C. Clotting took place in fourteen minutes, showing the presence of a proteolytic enzyme.

As the glands are small, it is necessary to dissect a large number of earthworms to obtain gland-extracts of appreciable strength. An idea of the extremely small weight of the gland can be gained from the fact that the glands of fifty earthworms dissected weighed only 0·884 gm., so that the gland in each earthworm weighs, on an average, only 0·0176 gm.

The next experiment was made to test the character of the enzyme in the glands.

Experiment 3.—Two hundred worms were dissected and their glands taken out. The glands weighed 3·4 gm. They were ground up in sand and 80 c.cm. of distilled water added, giving the strength of the gland-extract at 4·25 per cent. These extracts were kept at different temperatures and were allowed to digest their own substance for twenty-four hours. The amount of amino-acids produced was estimated as shown in the table on the next page.

The control was titrated first with formol solution and $\frac{N}{10}$ NaOH (phenolphthalein was used as indicator), and all other extracts were strictly compared on titration with the control. Toluol was employed as an antiseptic. The hydrogen-ion concentration of the gland-extract was tested with bromo-thymol blue and phenol red; the pH being 7·8.

This experiment clearly demonstrated the presence of a 'tryptic' enzyme. The best results were obtained between

34° and 40° C., which is very nearly the average temperature at Lucknow during August and September.

Serial No.	*Temp.*	*Experiment.*	$\frac{N}{10}$ *NaOH required.*
1	Control 33° C.	10 c.cm. extract + 2·5 c.cm. formol sol. (boiled)	0·15 c.cm.
2	Initial 0° C. Final 4° C.	,, ,,	0·15 c.cm.
3	Initial 10° C. Final 10° C.	,, ,,	0·2 c.cm.
4	Initial 20° C. Final 25° C.	,, ,,	0·3 c.cm.
5	Initial 30° C. Final 33° C.	,, ,,	0·6 c.cm.
6	Initial 40° C. Final 34° C.	,, ,,	0·7 c.cm.

This experiment was repeated several times with very nearly the same results. That the glands secrete a tryptic enzyme is, therefore, well established. The glands do not seem to act on starch and fats, as I have not been able to get a satisfactory test of their action on these substances.

A further piece of work affording strong evidence of the hepatic nature of these glands was carried out in the form of staining sections with Best's carmine to test the presence of glycogen granules. The glands were fixed in acetic absolute alcohol, and imbedded in celloidin-paraffin. The sections were stained with Best's carmine according to the technique given by Bolles Lee and Gatenby (4, 1921). Thickly set granules stained deep red were seen all along the secretory edges of the glandular-cells, clearly demonstrating the presence of glycogen granules in them (fig. 11, Pl. 10).

4. Discussion and Summary.

Students of the Oligochaeta are well aware of the fact that there is a great difference of opinion amongst various observers even with regard to the function of the calciferous glands. Michaelsen (6, 1895) holds strongly that the function of the calciferous glands is the absorption of nutriment, and he always designates them as 'chyle-sacs'. The excretion of lime, according to him, is a secondary function. The main objection urged against his view is the situation of the calciferous glands, since they lie far forward, and the main digestive region, i.e. the intestine, lies behind them. But this objection does not hold in the case of the intestinal glands, since they lie just at the right place, a little behind the middle of the body where the typhlosole ends, and the gut still extends behind for another 107 to 127 segments. They lie, therefore, just in the region where the main work of digestion and absorption takes place.

That the glands open into the gut by as many as eighteen openings in five segments strongly indicates that their secretions are poured into the gut through these openings. The nature of the secretions seems to be tryptic, since amino-acids were formed as products of digestion in the experiments made. Further, the greater part of the blood of the last portion of the intestine (107 to 127 segments) is collected and taken to these glands, where it permeates the substance of the gland through a sinusoid capillary network. The blood from the glands is collected again by the intestino-dorsals and taken to the dorsal vessel, the entire blood-supply of the glands resembling a hepatic portal system. These facts taken together with the demonstration of glycogen granules within the glandular cells strongly suggest that the intestinal glands are of the nature of a hepato-pancreas. The structure of the gland-cells themselves and the development of the glands further corroborate these conclusions.

The observations and conclusions arrived at may now be summarized:

1. The intestinal glands of Eutyphoeus waltoni lie on the dorsal surface of the gut and extend as paired glands from segments 79 to 83. As the two glands of a pair are fused in the middle dorsal line and the glands of successive segments are connected, we should speak of it as one large gland extending over five segments.

2. The gland forms the posterior boundary of the typhlosole of the gut, there being no typhlosole behind the region of the gland.

3. The gland is more or less solid and consists of lobules of glandular epithelium and interlacing lamellae. The lobules are separated from one another by sinusoid capillaries, while the two epithelial folds of a lamella enclose a blood-sinus between them.

4. The gland-cells are rhomboidal to polyhedral in outline, and their shape and structure strongly resemble those of liver-cells. They do not bear cilia or rodlets.

5. The whole gland opens into the gut through as many as eighteen apertures over five segments. These apertures are lined with the ciliated gut-epithelium.

6. The blood-supply of the gland resembles a hepatic portal system. The blood is collected from the gut of the last 107 to 127 segments into a ventral intestinal sinus which empties all its blood into the sinusoid capillaries of the gland. The capillaries of the gland join together to form five pairs of intestino-dorsal vessels which carry all the blood of the glands into the dorsal vessel.

7. The glands develop as dorsal outgrowths of the endodermal lining of the embryonic gut.

8. The glands secrete no calcium whatever. Calcified milk was curdled by the gland-extract in fourteen minutes. A tryptic enzyme has been demonstrated by the formation of amino-acids in digestion experiments.

9. Thickly set glycogen granules have been demonstrated by staining the gland-cells with Best's carmine.

10. The glands are, therefore, of the nature of a hepato-pancreas.

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EXPLANATION OF PLATES 9 AND 10.

All figures, except fig. 6, are of Eutyphoeus waltoni.

Plate 9.

Fig. 1.—A photograph of a dissection showing the hepato-pancreatic glands in situ. The segments are marked with their serial numbers. The intestino-dorsals form a pair of ring-like vessels going all round the gut in front of the glands, but are very short vessels behind the glands, just connecting the gut-sinus with the dorsal vessel. *b.w.*, body-wall; *d.v.*, dorsal vessel; *h.p.gl.*, hepato-pancreatic gland; *int.d.v.*, intestino-dorsal vessels; *i.s.*, intersegmental septum. (× about 3.)

Fig. 2.—Same as fig. 1, but the intestine has been cut open along one side and the flap reflected to show the structures on the inside. The typhlosole extends as a low ridge up to the seventy-eighth segment, but becomes expanded into a flat oblong structure to support the glands in segments 79 to 83. The ventral intestinal sinus is exposed and is seen dividing into the two lateral glandular sinuses which supply blood to the glands. The network of sinusoid capillaries is shown in the region of the glands. *d.v.*, dorsal vessel; *int.*, cut edge of the intestine; *int.d.v.*, intestino-dorsal vessels; *lat.gl.s.*, lateral glandular sinus; *n.*, diagrammatic representation of the network of sinusoid blood-capillaries in the glands; *ty*, typhlosole; *v.i.s.*, ventral-intestinal sinus.

Fig. 3.—A microphotograph of a transverse section passing through the hepato-pancreatic glands, which are seen to open into the lumen of the intestine through two contiguous openings (*O*). The capillaries of the glands are seen opening into the dorsal vessel. The lobules and lamellae of the gland and the sinusoid capillaries are clearly seen. *b.w.*, body-wall; *d.v.*, dorsal vessel; *h.p.gl.*, hepato-pancreatic gland; *int.*, intestine; *n.c.*, nerve-cord; *O.*, openings of the glands into the intestine; *v.v.*, ventral vessel. (× about 22.)

Fig. 4.—A camera-lucida drawing of a transverse section passing through the glands. *b.w.*, body-wall; *d.v.*, dorsal vessel; *h.p.gl.*, hepato-pancreatic glands showing lamellae and lobules and the sinusoid blood-capillaries shaded black; *lat.gl.s.*, lateral glandular sinus; *lat.n.v.*, lateral neural

vessel; *n.c.*, nerve-cord; *o.*, opening of the gland into the lumen of the gut; *v.v.*, ventral vessel. (× about 22.)

PLATE 10.

Fig. 5.—A camera-lucida drawing of a transverse section of the gland showing interlacing lamellae and lobules, and the rich blood-supply of the glands. The valves guarding the entrance of the blood from the glands into the dorsal vessel are also shown. *bl.sin.*, blood sinus; *cil.int.epith.*, ciliated intestinal epithelium; *d.v.*, dorsal vessel; *gl.duct.*, one of the ductules of the gland; *gl.duct.*^{2}, one of the large ducts of the gland; *int.d.v.*, short intestino-dorsal vessel in the region of the glands; *lat.gl.sin.*, lateral glandular sinus; *o.*, opening of the gland into the lumen of the intestine; *peri.epith.*, peritoneal epithelium; *v.*, valve guarding the entrance of the blood into the dorsal vessel. (× about 70.)

Fig. 6.—Beddard's diagram of the structure of the glands in Eutyphoeus gammii.

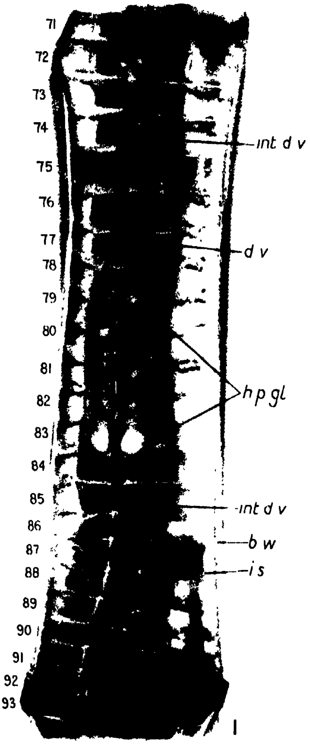
Fig. 7.—A camera-lucida drawing of a small portion of the gland showing lobules of the gland interspersed with sinusoid blood-capillaries. *bl.sin.*, sinusoid blood-capillary; *gl.c.*, gland-cell; *gl.duct.*, a ductule of the gland; *gl.lob.*, a gland-lobule. (× about 200.)

Fig. 8.—A transverse section passing through the post-typhlosolar intestine behind the region of the glands. The glands are absent, but the large ventral-intestinal sinus collecting blood in the posterior segments and supplying the glands therewith is prominently visible. *d.v.*, dorsal vessel; *lat.n.v.*, lateral neural vessel; *n.c.*, nerve-cord; *v.i.s.*, ventral intestinal sinus; *v.v.*, ventral vessel. (× about 25.)

Fig. 9.—A transverse section through the gut of an embryo showing the origin and development of the glands as mid-dorsal outgrowths from the wall of the gut. *d.v.*, dorsal vessel; *dor.div.*, dorsal diverticulum of the gut forming the glands; *end.*, endodermal lining of the gut; *int.gl.*, intestinal glands; *peri.ep.*, peritoneal epithelium. (× about 225.)

Fig. 10.—A transverse section passing through the glands at a more advanced stage of development than that shown in fig. 9. The mid-dorsal region of the gut shows proliferation of cells, and cells are also being budded from the endoderm on both sides. The peritoneal epithelium is also budding off cells which make their way into the gland-substance. *d.v.*, dorsal vessel; *gl.l.*, a gland-lobule; *int.end.*, intestinal endoderm full of albumen spheres; *m.d.r.*, mid-dorsal proliferating region of the gut with cells almost free of albumen; *peri.ep.*, peritoneal epithelium.

Fig. 11.—A microphotograph of a portion of a longitudinal section of the gland stained with Best's carmine to show the glycogen granules. The glycogen granules stained red (black in the photograph) are thickly aggregated along the secretory edges of the cells that line the ductules of the gland. *bl.c.*, blood-capillaries; *gl.d.*, ductules of the gland; *gl.g.*, glycogen granules.



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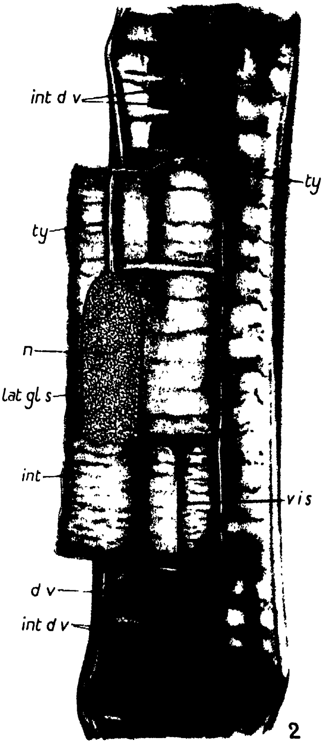

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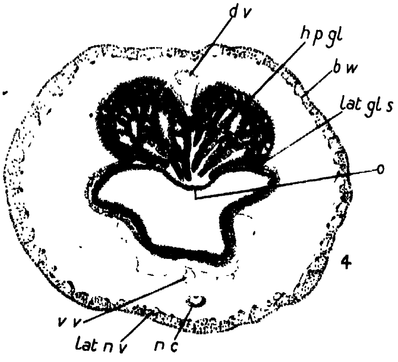

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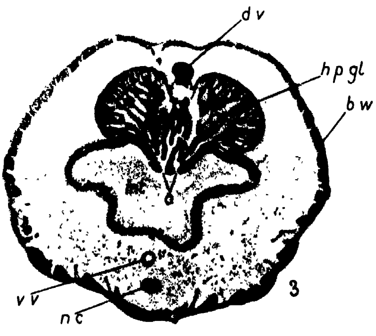

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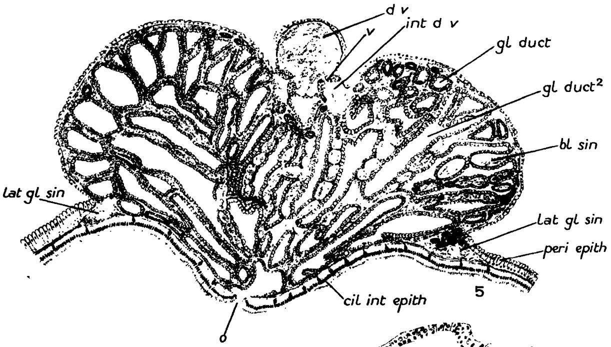

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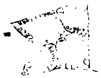

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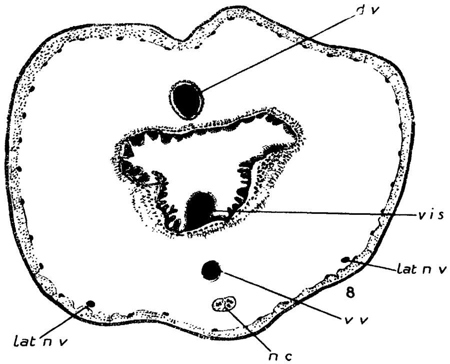

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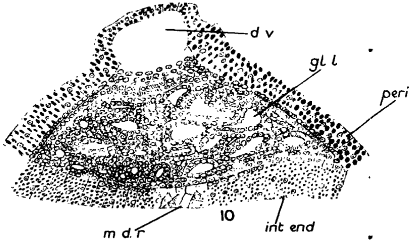

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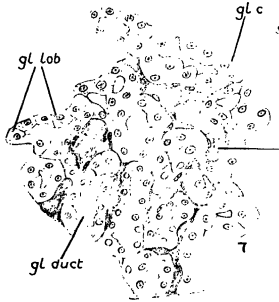

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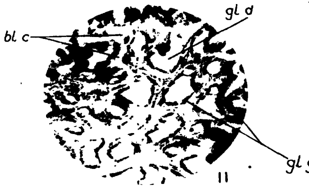

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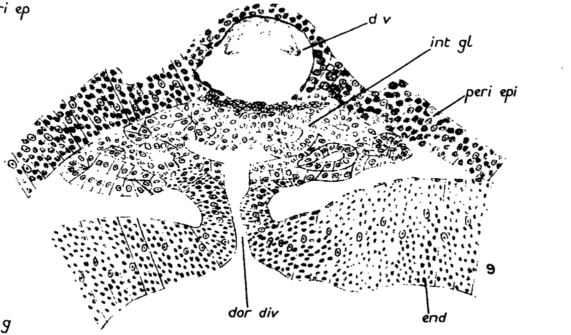

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K. N. Bahl and M. B. Lal.

Microchemical Tests for Fats, Lipoids, and Vacuoles with special reference to Oogenesis.

By

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INTRODUCTION

IF a substance goes black in osmic acid in a short time, for example, in twenty-four hours' immersion in Champy's fluid, and can be subsequently decolorized in turpentine, the cytologist is generally content to label it as fat. If, on the other hand, a substance goes black only after a prolonged period of osmication, for example, in four days' incubation in 2 per cent. osmic acid at 40° C., and cannot be subsequently decolorized by turpentine, he complacently considers it as lipoidal in nature, and assigns it to the category of Golgi apparatus. Observations which are recorded in this paper prove that the above test is by itself very unsatisfactory. Its exclusive use by the majority of cytologists has resulted in much confusion.

The time taken by a particular sample of fat or lipoid to blacken in osmic acid depends entirely on the degree of unsaturation, and the amount of oxidation it has already undergone. Recently a sample of completely hydrogenized fat, whose iodine value was nil, and which had been prepared from whale fat, was supplied to me through the courtesy of Dr. J. N. Ray of the Punjab University Chemical Laboratory. Films of this completely saturated fat did not blacken in the slightest degree, even in seven days' incubation in 2 per cent. osmic acid at 40° C. It is, however, very uncertain whether completely saturated fats and lipoids exist in such a substance as protoplasm which is in a state of continual chemical flux. Similarly Walker and Allen (1927) failed to blacken in osmic acid methyl myristate, which is another example of a completely saturated fat. Thus it is clear that samples of fat can be found which will actually take longer time to blacken in osmic acid than many samples of lipoids, simply because the latter are more unsaturated than the former. It follows, therefore, that osmic acid is an unsuitable test for distinguishing fats from lipoids.

There are of course other tests which have been discussed by Gatenby and Cramer in Lee's 'Vade Mecum', to which reference may be made. One of these seems trustworthy, and at the same time is highly convenient to employ. This is the Sudan III or Scharlach R test. True fats are stained deeply with these dyes, whereas the majority of lipoids are not. Lipoidal substances such as cholesterin-esters and cholesterin fatty acid mixtures also stain, but much less intensely. This test again seems to be uncertain; but for the purposes of the thesis developed in this paper it is enough that true fats, according to Gatenby and Cramer, always stain deeply. In other words, if a granule in the cell is not stained with Sudan III and Scharlach R, it is not fat.

The classical lipoidal Golgi apparatus has been blackened after a short treatment with osmic acid. Bowen (1919 and 1928) succeeded in blackening the Golgi apparatus in the testis of Hemiptera and other insects after twenty-four hours' immersion in Mann's corrosive osmic mixture. Weigl (1910, as quoted by Bowen) found that after five to ten minutes' exposure to 2 per cent. osmic acid at 25° C. some traces of the Golgi apparatus (presumably in somatic cells) were just barely visible. After one hour the blackening is more obvious. Similarly Nath has demonstrated the Golgi apparatus in several eggs after short periods of osmication. This has led Harvey (1931) to state that the material which Nath and his co-workers have described as Golgi in several eggs is in reality fat. He thinks he has proved in the case of the earthworm that the Golgi spherules of Gatenby and Nath (1926, Lumbricus) and of Nath (1930, Pheretima) are droplets of fat. I welcomed this statement because it has induced me to use fat tests which I had not so far used, except in one or two cases.

Staining with Sudan III and Scharlach R has proved that not only in the earthworm egg but in all other eggs worked out by Nath and his co-workers, the substance described as Golgi apparatus material is anything but fat, and it is only in later stages of oogenesis in some eggs that it is converted into fat. Harvey (1931 *a*) has admitted that in Antedon the Golgi elements go dark after ten to twenty minutes' immersion in 1 per cent. osmic acid.

At the end of this paper a chart is published recording the reactions of fats, lipoids, and vacuoles to various microchemical tests employed.

MATERIAL AND METHODS

Ovaries are kept overnight in saturated alcoholic (90 per cent. alcohol) solutions of Sudan III and Scharlach R either directly or after fixation in formalin (formol 10 c.c., H_2O 50 c.c.). The results with both the dyes are absolutely identical. The material is brought down through lower grades of alcohol to water or glycerine in which they are studied. There is no danger of washing out these stains in this process because they are not at all soluble in grades of alcohol lower than 90 per cent.

On account of the presence of a large amount of accessory tissue covering the oocytes this technique could not be employed in the case of the rabbit. In the absence of a freezing microtome the following technique was employed: Ovaries are fixed in formalin. After a wash in water they are first transferred to 90 per cent. alcoholic solutions of the dyes and then to their solutions in absolute alcohol. They are cleared in cedar-wood oil and embedded in paraffin in the usual way. Sections are mounted in Canada balsam after removing the paraffin by xylol.

These experiments have been carried out on eggs of animals representing the following groups: Fishes, Amphibia, Reptiles, Birds, Mammals, Insects, Crustaceans, Spiders, and Annelids. The scolopendrid Otostigmus, on the oogenesis of which Nath and Husain published a paper in 1928, could not be collected from Lahore, and no work, therefore, could be carried out on this material.

OBSERVATIONS.

Fishes (Rita rita and Ophiocephalus punctatus).

Nath and Nangia (1931) have already reported that the Golgi elements of young oocytes of Rita (oocytes measuring more than 0·5 mm. were not available for study) are not fatty. They do not go black in Champy's fluid and they required thirty-two days to be impregnated in Mann-Kopsch. Repeated trials with Kolatschev were negative, although the eggs were incubated in 2 per cent. osmic acid for ten days at 38° C. They went black in Da Fano.

Recently it has been possible to obtain older oocytes of Rita. Staining with Sudan III and Scharlach R shows that there is fatty yolk in this egg, that it appears when the egg measures about 1 mm., and that in younger eggs the Golgi elements are not at all coloured.

In Ophiocephalus, according to Nath and Nangia (1931), the Golgi elements of early oocytes are non-fatty, and become black, not only in Kolatschev and Da Fano, but also after forty-eight hours' osmication at room temperature. Gradually they grow in size, become fatty and form the fatty yolk which in eggs measuring 1 mm. goes brilliantly red in Sudan III.

Younger oocytes measuring up to 0·375 mm. have been recently stained with Sudan III and Scharlach R and it has not been found that red granules appear in the cytoplasm. It has not been possible to obtain oocytes measuring anything between 0·375 mm. and 1 mm., so that it is unknown at what stage the Golgi elements become fatty.

Amphibia (Rana tigrina and Rana cyanophlyctis).

Nath (1931) has already reported that in Rana tigrina the Golgi elements remain non-fatty throughout oogenesis inasmuch as they cannot be stained with Sudan III. They are blackened not only in Da Fano and Kolatschev but also in Champy. It must be noted that oocytes measuring more than 1·08 were not studied.

Recently it has been possible to stain oocytes of all stages both with Scharlach R and Sudan III. It is found that the Golgi elements stain with these dyes only when the oocyte reaches 1·2 mm. in size. The biggest egg of this species studied by me measures 1·5 mm.

In Rana cyanophlyctis, on the other hand, the Golgi elements begin to stain with these dyes at a much earlier stage when the egg measures a little more than 0·5 mm.

Reptiles (Emyda granosa).

In the oocytes of this tortoise the Golgi elements begin to stain with Sudan III and Scharlach R only when the egg has grown to about 0·6 mm. Work on the oogenesis of this species has been completed in this laboratory by Aziz Ahmad, and will be published in due course. It will suffice to mention here that the Golgi elements of this species go black not only in Da Fano and Kolatschev but in Champy also, even in the earliest oocytes.

Birds (Gallus bankiva).

In the earliest oocytes in which the Golgi elements are in the juxta-nuclear position, they are blackened even in Champy, although they tend to get decolorized after mounting. They can be blackened in Kolatschev throughout oogenesis. Experiments with Sudan III and Scharlach R revealed that there is variation in the time of the appearance of fatty yolk. Oocytes measuring 1 mm. sometimes show red granules and sometimes not. However, oocytes measuring 0·2 mm. never showed any red granules. It has not been possible to obtain oocytes measuring anything between 1 mm. and 0·2 mm.

Mammals (Rabbit).

The oogenesis of this animal has been worked out in this laboratory by Sukh Dyal, and his results will be published shortly. It will suffice here to mention that the Golgi elements of this species go black even in Champy, although they tend to get decolorized after mounting. They are blackened in Kolatschev and Da Fano. But they do not go red in Sudan III and Scharlach R, even in the slightest degree throughout the whole oogenesis.

Insects (Luciola gorhami, Periplaneta americana, Culex fatigans, and Dysdercus cingulatus)

In Luciola, according to Nath and Mehta (1929), the Golgi vesicles of even the primordial germ-cells contain some quantity of fat, inasmuch as they are blackened in short periods of osmication. In the course of oogenesis they swell up, become more fatty, and form the fatty yolk of the egg. They go jet-black in Mann-Kopsch like the typical Golgi apparatus.

As the result of experiments with Sudan III and Scharlach R, it appears that the Golgi elements become fatty only when the egg measures about 0·6 mm. At this and later stages they stain brilliantly with these dyes, but in the younger oocytes, in the undifferentiated germ-cells, and in the follicular epithelium, they are not coloured even slightly.

According to Nath and Piare Mohan (1929) the Golgi vesicles in the youngest oocytes of Periplaneta americana hardly contain any fat as they do not go black in osmic acid in twenty hours or even in forty-eight hours. Early in oogenesis, however, they become fatty and give rise to the fatty yolk. They are blackened in Mann-Kopsch, Kolatschev, and Da Fano like the typical Golgi apparatus. These conclusions and others have now been confirmed in every detail by Gresson (1931), who has worked on the allied species, Periplaneta orientalis.

Experiments with Scharlach R and Sudan III show that the Golgi vesicles do not show the slightest amount of colour in eggs measuring 1·675 mm. or less. When, however, the egg measures 3 mm. the Golgi vesicles go brilliantly red. No oocytes measuring anywhere between 1·675 mm. and 3 mm. were obtainable.

In Culex, according to Nath (1929), the Golgi vesicles of the youngest follicle, in which the oocyte is not differentiated from the nurse-cells, are not fatty. They react to Mann-Kopsch and Da Fano like the typical Golgi apparatus. The contents of the Golgi vesicles of the nurse-cells and the follicular epithelial cells remain non-fatty throughout the growth period, while those of the majority of the oocyte vesicles become fatty, inasmuch as they go black in Champy, even though they do not grow in size.

Repeated treatment of fresh as well as of formol-fixed ovaries with Scharlach R and Sudan III has completely failed to stain the Golgi vesicles of the egg during all stages of oogenesis. The same is true of the vesicles of the nurse-cells and the follicular epithelial cells.

In Dysdercus, according to Bhandari and Nath (1930), the Golgi vesicles of even the earliest oogonia contain some fat inside them, as they go black in osmic acid in short periods. They react to Kolatschev like the typical Golgi material. In the course of oogenesis many of them swell up, become more fatty, and give rise to the fatty yolk.

Repeated treatment of fresh and formol-fixed ovaries with Scharlach R and Sudan III produced interesting results. Even in eggs measuring about 1 mm. the Golgi elements are not at all coloured. The size of the 'ripe' egg of this species is not known, but the writer has never seen eggs which are bigger than about 1 mm. It may be that there are bigger eggs, and in these the swollen Golgi elements (fatty yolk) are stainable with these fat dyes.

Crustaceans (Palaemon lamarrei and Paratelphusa spinigera).

In Palaemon, according to Bhatia and Nath (1931), the non-fatty Golgi vesicles of the youngest oocyte grow in size and become fatty in the course of oogenesis, forming the fatty yolk of the egg.

Experiments with Scharlach R and Sudan III show that the Golgi vesicles are coloured with these dyes only in oocytes measuring more than 0·15 mm. Similarly in the fresh-water crab, Paratelphusa spinigera, the Golgi vesicles cannot be stained with these dyes in oocytes measuring less than 0·6 mm., although they go dark in osmic acid in four hours.

Spiders (Crossopriza lyoni and Plexippus paykulli).

In Crossopriza, according to Nath (1928), the Golgi vesicles are non-fatty in the youngest oocytes, but in later stages they give rise to the fatty yolk. They go black in Mann-Kopsch.

The laid eggs of this species measure between 0·7 and 0·8 mm., but the ovarian oocytes are not known to measure more than 0·6 mm. Scharlach R and Sudan III have failed to stain the Golgi elements of ovarian eggs, even though they can be

	a, b, c; for explanation see under remarks on right.	*Kolatschev or Mann-Kopsch prolonged osmication.*	*Champy or Flemming without acetic for 24 hours.*	*Immersion in 2 per cent. osmic acid (whole mounts or contents studied after rupturing).*
Ophiocephalus punctatus.	(*a*) (*b*) (1 mm. possibly earlier; absent up to 0·375 mm. stage) (*c*)	(*a*) Black. (*b*) Black. (*c*) White spaces with a yellow yolk inside in older oocytes.	(*a*) Black. (*b*) Black. (*c*) Same as for Kolatschev.	(*a*) Dark in 48 hours in youngest oocytes but earlier in older. (*b*) Black. (*c*) Slightly yellowish.
Rita rita.	(*a*) (*b*) (1 mm.) (*c*)	(*a*) Black. (*b*) *, but should be black. (*c*) White empty spaces.	(*a*) Not blackened. (*b*) Same as for Kolatschev. (*c*) White empty spaces.	(*a*) Not blackened. (*b*) Black. (*c*) Slightly yellowish.
Rana tigrina.	(*a*) (*b*) (1·2 mm.) (*c*)	(*a*) Black. (*b*) Black. (*c*) White empty spaces.	(*a*) Black. (*b*) Black. (*c*) Same as for Kolatschev.	(*a*) Dark within 10 minutes. (*b*) Black. (*c*) Slightly yellowish.
Rana cyanophlyctis.	(*a*) (*b*) (0·5 mm.) (*c*)	(*a*) Black. (*b*) Black. (*c*) White empty spaces.	(*a*) Black. (*b*) Black. (*c*) Same as for Kolatschev.	(*a*) Dark within 10 minutes. (*b*) Black. (*c*) Slightly yellowish.
Emyda granosa.	(*a*) (*b*) (0·6 mm.) (*c*)	(*a*) Black. (*b*) Black. (*c*) White empty spaces.	(*a*) Black. (*b*) Black. (*c*) White empty spaces.	(*a*) Dark. (*b*) Black. (*c*) *
Gallus bankiva.	(*a*) (*b*) (1 mm. possibly earlier; absent up to 0·2 mm. stage) (*c*) (absent)	(*a*) Black. (*b*) Black.	(*a*) Black. (*b*) Black.	(*a*) Dark. (*b*) Black.
Lepus cuniculus.	(*a*) (*b*) (absent) (*c*)	(*a*) Black. (*c*) Small empty spaces.	(*a*) Black. (*c*) Small empty spaces.	(*a*) * (*c*) *
Dysdercus cingulatus.	(*a*) (*b*) (absent) (*c*) (absent)	(*a*) Black.	(*a*) Black.	(*a*) Dark within 10 minutes.
Culex fatigans.	(*a*) (*b*) (absent) (*c*) (absent)	(*a*) Black.	(*a*) Black.	(*a*) Dark within 2 hours.
Luciola gorhami.	(*a*) (*b*) (0·6 mm.) (*c*) (absent)	(*a*) Black. (*b*) Black.	(*a*) Black. (*b*) Black.	(*a*) Dark within 10 minutes. (*b*) Black.
Periplaneta americana.	(*a*) (*b*) (3 mm. possibly earlier; absent up to 1·675 mm. stage) (*c*) (absent)	(*a*) Black. (*b*) Black.	(*a*) Black. (*b*) Black.	(*a*) Dark in 48 hours. (*b*) Black.
Palaemon lamarrei.	(*a*) (*b*) (0·15 mm.) (*c*) (absent)	(*a*) Black. (*b*) Black.	(*a*) Black. (*b*) Black.	(*a*) Dark in 22 hours. (*b*) Black.
Paratelphusa spinigera.	(*a*) (*b*) (0·6 mm.) (*c*) (absent)	(*a*) Black. (*b*) Black.	(*a*) Black. (*b*) Black.	(*a*) Black. (*b*) Black.
Crossopriza lyoni.	(*a*) (*b*) (absent) (*c*) (absent)	(*a*) Black.	(*a*) Black.	(*a*) Black.
Plexippus paykullii.	(*a*) (*b*) (absent) (*c*) (absent)	(*a*) Black.	(*a*) Black.	(*a*) Black.
Pheretima posthuma.	(*a*) (*b*) (absent) (*c*) (absent)	(*a*) Black.	(*a*) Black.	(*a*) Black.

Da Fano's silver nitrate technique.	*Sudan III and Scharlach R over-night after formol-fixation.*	*Neutral red.*	*Intra vitam.*	*Remarks.*
(a) Black. (b) *, but should appear as empty spaces. (c) White empty spaces with a whitish coagulum in older oocytes.	(a) Not coloured. (b) Deep red. (c) Not coloured.	(a) Not coloured. (b) Not coloured. (c) Red.	(a) From 0·2 mm. stage as dark-greyish refractile bodies. (b) Refractile spheres. (c) Whitish droplets.	I. (a) = Golgi elements. (b) = Fat. (c) = Vacuoles. II. Measurements within brackets against (b) indicate the size of the egg when fat appears for the first time. III. * indicates the observation with the particular technique is lacking. IV. A glance at this chart will at once show that neither the Golgi material nor the fats stain with neutral red. But the fatty yolk of the Scolopendrid Otostigmus has been reported by Nath (1928) to stain with this dye. This was based on a single highly advanced oocyte and should be taken with reserve. It seems likely that late in oogenesis watery vacuoles appear in the egg which might have stained with neutral red.
(a) Black. (b) As in Ophiocephalus. (c) White empty spaces.	(a) Not coloured. (b) Deep red. (c) Not coloured.	(a) Not coloured. (b) Not coloured. (c) Red.	(a) Very refractile granules with definite bluish tinge. (b) Refractile spheres. (c) Whitish.	
(a) Black. (b) *, but should appear as empty spaces. (c) White empty spaces.	(a) Not coloured. (b) Deep red. (c) Not coloured.	(a) Not coloured. (b) Not coloured. (c) Red.	(a) Refractile granules dark-greyish. (b) Very refractile spheres. (c) Slate-coloured.	
(a) Black. (b) * (c) White empty spaces.	(a) Not coloured. (b) Deep red. (c) Not coloured.	(a) Not coloured. (b) Not coloured. (c) Red.	(a) Refractile granules dark-greyish. (b) Very refractile spheres. (c) Whitish.	
(a) Black. (b) Empty spaces. (c) Empty spaces.	(a) Not coloured. (b) Deep red. (c) Not coloured.	(a) Not coloured. (b) Not coloured. (c) *	(a) * (b) Very refractile spheres. (c) *	
(a) Black. (b) Empty spaces.	(a) Not coloured. (b) Deep red.	(a) Not coloured. (b) Not coloured.	(a) Refractile granules dark-greyish. (b) Very refractile spheres.	
(a) Black. (c) Small empty spaces.	(a) Not coloured. (c) Not coloured.	(a) * (c) *	(a) * (c) *	
(a) Black.	(a) Not coloured.	(a) Not coloured.	(a) Refractile greyish spheres.	
(a) Black.	(a) Not coloured.	(a) Not coloured.	(a) Dark-greyish granules.	
(a) * (b) *	(a) Not coloured. (b) Deep red.	(a) Not coloured. (b) Not coloured.	(a) Dark-greyish. (b) Refractile spheres.	
(a) Black. (b) Empty spaces.	(a) Not coloured. (b) Deep red.	(a) Central portion stains 'very slightly' but the cortex is dark-greyish. (b) Not coloured.	(a) Dark-greyish. (b) Refractile spheres.	
(a) Black. (b) Empty spaces.	(a) Not coloured. (b) Deep red.	(a) Not coloured. (b) Not coloured.	(a) Cannot be seen. (b) Refractile spheres.	
(a) Black. (b) Empty spaces.	(a) Not coloured. (b) Deep red.	(b) Not coloured. (b) Not coloured.	(a) * (b) *	
(a) Black.	(a) Not coloured.	(a) Not coloured.	(a) Dark-greyish.	
(a) Black.	(a) Not coloured.	(a) Not coloured.	(a) Dark-greyish.	
(a) Black.	(a) Not coloured.	(a) Not coloured.	(a) Dark-greyish.	

blackened in Champy's fluid in twenty-four hours. Identical results have been obtained in Plexippus.

Annelids (Pheretima).

Fresh ovaries are transferred directly into alcoholic solutions of Sudan III and Scharlach R and kept there for varying periods. Nothing whatsoever in the oogonia or in the oocytes of any stage is tinged even slightly by these dyes. The Golgi spherules are slightly corroded, but they still appear as dark greyish vesicles as in the fresh material. After fixation in formalin and treatment with Sudan III and Scharlach R for varying periods exactly the same result is obtained, except that the Golgi spherules are not distorted. The period of immersion in Scharlach R was extended to a fortnight, but the Golgi spherules remained absolutely unaffected by the stain.

In Lumbricus, Harvey reports that fat is present in the egg, that it gives a negative reaction to one fat test (Nile blue sulphate), a positive one to another (Scharlach R), and a weak one to another (Sudan III). This is difficult to believe, and I regard his recent interpretations of the Lumbricus oogenesis as very doubtful.

DISCUSSION.

The most important conclusion of the present investigation is that if a granule goes black in short periods of osmication it must not be labelled as fat unless it also goes red in Sudan III and Scharlach R. These two dyes stain brilliantly all true fats, whereas osmic acid will quickly blacken lipoids also if they are highly unsaturated or are very little oxidized.

In Pheretima, Culex, Dysdercus, Crossopriza, Plexippus, and the rabbit the substance that I have described as Golgi material goes black in Da Fano and Kolatschev or Mann-Kopsch. But it also goes black, not only in Champy in twenty-four hours, but in much shorter periods of immersion in 2 per cent. osmic acid. In the youngest, as well as in the most advanced oocytes studied, it remains absolutely uncoloured after treatment with Sudan III and Scharlach R. It must, therefore, be interpreted as a material which consists of highly unsaturated or very little oxidized lipoid.

In Ophiocephalus, Rana, Emyda, Gallus, Luciola, Periplaneta, Palaemon, and Paratelphusa the substance described as Golgi material reacts to Da Fano, Kolatschev, or Mann-Kopsch like the typical Golgi apparatus, and also goes dark in short periods of osmication. In early stages of oogenesis, it is not stained in the slightest degree with Scharlach R and Sudan III and must again be interpreted as highly unsaturated or very little oxidized lipoid. But at some stage in oogenesis (see observations for exact measurements) these lipoids are converted into fats when they stain brilliantly with Scharlach R and Sudan III. They may now be truly described as the fatty yolk of the egg.

Lastly, in Rita the Golgi material of early oocytes consists of very nearly fully saturated lipoids, as it does not go black at all in Champy in twenty-four hours or in shorter periods of osmication, and takes thirty-two days to impregnate in Mann-Kopsch. It does not stain with Sudan III and Scharlach R, but by the time the egg measures 1 mm. it is converted into fat and begins to stain brilliantly with these dyes.

Harvey (1931 *a*) suspects that Nath may not be dealing with a Golgi apparatus at all, but with developing fatty droplets. In a way Harvey's suspicion is justified because biochemists tell us that lipins form fat in the cell (Hammersten, 1904; Maclean and Williams, 1909; and Heffter, 1891. Quoted by Walker and Allen, 1927), and the lipoidal Golgi elements of many eggs (Rita, Ophiocephalus, Rana, Emyda, Gallus, Luciola, Periplaneta, Palaemon, and Paratelphusa) may aptly be described as 'developing fatty droplets'. Harvey (1931 *a*) has himself reported that in the accessory cells of the ovary of Asterias, fat arises under the influence of Golgi bodies. Among the recent supporters of the view that the lipoidal Golgi apparatus forms fat in the cell may be cited Gresson (1929, 1929 *a*, and 1931) and Bell (1929). Gresson has confirmed in every detail Nath and Piare Mohan's account of the origin of fatty yolk from the Golgi apparatus in the egg of the cockroach, and Bell has shown that even in the male germ-cells fat may be derived from the same source.

Apart from the tests described in the introductory pages of this paper, there are other ways of distinguishing fat globules from the Golgi material. The juxta-nuclear or the circum-nuclear arrangement of the Golgi elements in the earliest oocytes and their subsequent orderly dispersal are of great diagnostic value. The presence of exactly similar material in the tiny undifferentiated germ-cells and in the follicular epithelia is still another point against regarding them as inert globules of fat.

Recent research from this laboratory has clearly demonstrated that in the eggs of Rita rita, Ophiocephalus punctatus, and Rana tigrina (see Nath, 1931; and Nath and Nangia, 1931) watery neutral red-staining vacuoles (Parat's vacuome) exist independently of the classical lipoidal Golgi apparatus, and that in Ophiocephalus these vacuoles condense albuminous yolk in their interior as claimed for Perca and Pygosteus by Hibbard and Parat (1928), and for Discoglossus by Hibbard, 1928. Similarly in the egg of the tortoise Emyda granose (Aziz Ahmad, unpublished) the vacuoles and the Golgi apparatus are independent cytoplasmic components. A series of papers (for references see Nath, 1931) on the germ-cells of both sexes, on the somatic cells, and on plant-cells also have now conclusively proved that the vacuome of Parat is not the Golgi apparatus. So that the claim of Parat that the Golgi apparatus (his vacuome) gives rise to albuminous yolk in eggs and to aleurone grains in plants is shown to be incorrect.

So far as I am aware Harvey (1931 *a*, in Carcinus, Antedon, and Asteries) and Weiner (1925 in Tegenaria and Lithobius) are the only workers who claim that proteid yolk is formed by the lipoidal Golgi apparatus. No evidence has been discovered for this view by Bhatia and Nath (1931), in such crustaceans as Palaemon and Paratelphusa, by King (1926), in the isopod Oniscus, by Hilton (1931), in the copepod Calanus, by Nath (1928), in the spider Crossopriza, by Bambeke (1898), in the spider Pholcus, and by Sukh Dyal and Nath (in press), in the spider Plexippus paykulli, whose so-called 'yolk-nucleus' is exactly like that of Tegenaria as described by Weiner.

With regard to Lithobius, I have recently had an opportunity of studying Weiner's brief account through the courtesy of Professor Bhattacharya who kindly sent me the original paper. I was interested to discover that Weiner does not make any mention of nucleolar extrusions which are so big and prominent in this egg. The fact is that he never made the necessary control preparations (e.g. Bouin and iron-haematoxylin) which very clearly demonstrate the remarkable process of nucleolar budding in this material. Weiner describes groups of Golgi elements scattered in the cytoplasm, each group being embedded in a grey field (his 'Champs gris'). This grey field later becomes rounded and vacuolated with the Golgi elements lying on its surface. This is described as the yolk-nucleus. As I will show in detail in a separate publication, the 'Champs gris' of Weiner is nothing but the slightly darkened cytoplasm in which the Golgi elements are embedded and his 'yolk-nucleus' is nothing but a big vacuolated nucleolar extrusion, the presence of Golgi elements on the surface of which is merely fortuitous. Reference may be made to Nath's figures 6 and 12 of nucleolar extrusions which have been figured as vacuolated round bodies exactly like the 'yolk-nucleus' of Weiner (see Nath, 1924).

Recent research has very clearly shown that the Golgi apparatus, like the mitochondria, is polymorphic. It may exist in the form of a granule, a ring or a vesicle, a crescent, a dictyosome, or a platelet, and finally in the form of network. Very often it has a duplex structure, showing an osmiophilic and an osmiophobic part. The former is generally understood to be lipoidal in nature, although direct evidence to support this view is still lacking. The latter has been interpreted by some as a specialized piece of cytoplasm and by others (including myself) as a vacuole. I am now inclined to accept neither of these views. A more rational view is that the osmiophilic part of the Golgi element consists of unsaturated lipoids and the osmiophobic part of very nearly fully saturated lipoids. This view is not only in accord with the well-known osmic acid reaction, but also easily explains the absence of the chromophobic part in the granular and the network type of Golgi apparatus. It also explains those cases in which attempt after attempt has failed to impregnate the Golgi apparatus. Here we have simply to suppose that the Golgi material is so nearly fully saturated, or is so nearly fully oxidized, that it does not take up any oxygen from osmic acid.

SUMMARY.

1. It is generally believed by cytologists that fats blacken quickly in osmic acid, whereas lipoids take a much longer time. This view seems to be erroneous, as the time required by osmic acid to blacken a particular sample of fat or lipoid depends entirely on its degree of unsaturation and its previous state of oxidation.
2. Films of a sample of completely hydrogenized fat, with iodine value nil, failed to blacken in osmic acid, even in seven days at 40° C. Samples of fat, therefore, must exist which actually take longer time to blacken in osmic acid than some highly unsaturated lipoids.
3. It follows, therefore, that a granule in the cell, which goes black in osmic acid in a short period, cannot be identified as true fat unless it also stains intensely in Sudan III and Scharlach R.
4. These fat tests have been employed on eggs of animals representing many groups.
5. In Pheretima, Culex, Dysdercus, Crossopriza, Plexippus, and the rabbit, the Golgi material can be blackened in short periods of osmication, but it cannot be stained even slightly with Sudan III and Scharlach R during any stage in oogenesis. It must, therefore, be interpreted not as fat but as very highly unsaturated or very little oxidized lipoid.
6. In earlier stages of the oogenesis of Ophiocephalus Rana, Emyda, Gallus, Luciola, Periplaneta, Palaemon, and Paratelphusa, the Golgi material does not stain at all with Sudan III and Scharlach R, even though it can be blackened in short periods of osmication. It must again be interpreted, not as fat, but as highly unsaturated or very little oxidized lipoid. At a certain stage in oogenesis these

lipoids are converted into fats when they stain brilliantly with Scharlach R and Sudan III.

7. In Rita the Golgi material of early oocytes consists of very nearly fully saturated lipoid, as it does not go black in short periods of osmication, and takes thirty-two days to impregnate in Mann-Kopsch. It does not stain with Sudan III and Scharlach R, but by the time the egg measures 1 mm. it is converted into fat and begins to stain intensely with these dyes.

8. A chart is published recording the reactions of fats, lipoids, and vacuoles to various microchemical tests.

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Protozoa from Australian Termites.

By

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With 9 Text-figures.

Very little work has been done on the protozoa of Australian termites except for the observations of Grassi (1917), who described the faunas of Porotermes adamsoni Frogg., Coptotermes lacteus Frogg., and a species of Schedorhinotermes, and those of Duboscq and Grassé (1927), who worked on Calotermes iridipennis Frogg.

In this paper it is proposed to give, first, a list of those flagellates previously found in termites of other countries which are now found to be present in Australian hosts; and, second, descriptions of four new genera and three new species of Protozoa parasitic in termites.[1]

Material was obtained from many parts of Victoria, from New South Wales, Western Australia, and North Queensland (Ayr). It was found that termites usually lived well under laboratory conditions, kept in glass or tin vessels, supplied with a small amount of moisture from damp filter-paper on the roof of the container, and fed either on the wood in which they were found, or else, for better detailed examination of the Protozoa, on filter-paper.

Technique.—Living Protozoa were examined in a drop of saline, 0·75 per cent. strength being used for Devescovinids, while a concentration of 0·4 per cent. was found most suitable for Trichonymphids. Cleveland's medium (1925) for Trichonympha campanula was tried for other Trichonymphids, but without marked success. Examination in water was sometimes useful; thus in Spirotrichonympha spp. the counting of the flagellar bands was made easier by the distension of the animal's body in water.

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[1] The work summarized in this paper was carried out in the Zoology Department of the University of Melbourne, while holding a Howitt Research Scholarship.

I have to thank Dr. G. Buchanan for her constant assistance and encouragement throughout the year, and Dr. Tiegs for his help in interpreting many of the more intricate histological details.

My thanks are also due to Mr. G. F. Hill of the Council for Scientific and Industrial Research, Canberra, who has both provided and identified many termites; and to many others who have collected material in various parts of Australia.

Division stages of the flagellates were occasionally obtained by changing the diet of the termite from wood to filter-paper or to cotton-wool and mud as suggested by Koidzumi (1921), or by starvation for 5–7 days followed by feeding.

The woody nature of ingested food-particles was demonstrated by the use of phloroglucin and HCl.

Attempts were made to culture the organisms, both in liquid media, e.g. bouillon with and without powdered wood, and on solid media, e.g. cellulose agar, maltose agar, Czapek's agar and serum agar+powdered wood, but all these proved unsuccessful.

Permanent preparations, made as wet films, were fixed with hot or cold Schaudinn's fluid, with corrosive acetic, Bouin's fluid, or osmic acid vapour. Osmo-chromic fixative was used for those smears where detailed cytology was desired. Smears were stained with Delafield's haematoxylin, Heidenhain's iron haematoxylin (watery or alcoholic) or Picro-carmine, after osmic fixation. Giemsa's stain was used for some smaller flagellates, and some preparations of termite spirochaetes were made as dry films and stained with Damon's mixture of aniline gentian violet and carbol fuchsin (1926). Sections of alimentary canals, dissected out and fixed in Bouin or hot corrosive acetic, were stained with Delafield or iron haematoxylin and eosin.

RECORDS.

1. From Porotermes grandis Holmgr.

Hill (1926) has considered that Porotermes grandis Holmgr. is probably a larger variety of Porotermes adamsoni Frogg. and should not be ranked as a separate species. An examination of its protozoan fauna reveals the following facts:

(*a*) All the genera and species of protozoa described by Grassi (1917) from Porotermes adamsoni—namely Trichonympha magna, Joenina pulchella, Spirotrichonympha mirabilis, Spirotrichonymphella pudibunda, and Pseudotrypanosoma giganteum—are present in Porotermes grandis.

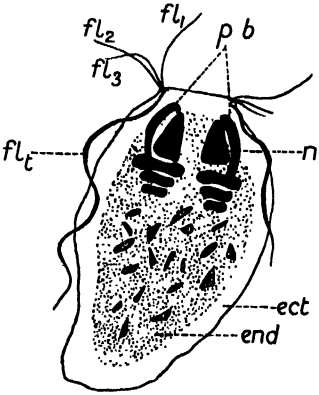
(*b*) In addition to these, there is a new species of Spirotrichonympha, which I have called Spirotrichonympha grandis, and a new species of Pseudotrypanosoma—Pseudotrypanosoma minimum—not recorded, from Porotermes adamsoni.

The presence, both in Porotermes adamsoni and Porotermes grandis, of numerous small forms of Spirotrichonymphella pudibunda, together with the typical 'adult' forms and many intermediate in size, would suggest the occurrence of multiple fission in this species.

2. From Calotermes (Neotermes) insularis White.

The two most abundant organisms are probably identical with Stephanonympha silvestrii and Devescovina striata var. hawaiensis, both of which were described by Janicki (1915) from a termite which he called Neotermes castaneus Burm., but which Kirby (1926) has identified as Neotermes connexus Snyder. Like Spirotrichonymphella pudibunda from Porotermes adamsoni, Stephanonympha silvestrii is densely invested with adherent spirochaetes and bacteria. Cleveland (1928) finds that these organisms are detached by feeding the termite on filter-paper impregnated with acid fuchsin. My own experiments have confirmed this in both species. But whereas Spirotrichonymphella pudibunda continues to exhibit unimpaired activity after this process, Stephanonympha silvestrii invariably died after detachment of its adherent organisms, suggesting that a closer relation may exist between them and their carrier in Stephanonympha silvestrii than in Spirotrichonymphella pudibunda.

In Janicki's description of Devescovina striata, he states that the 'stout form' having a parabasal tube of 3–4 coils is probably merely that stage in the life history of the 'slender form' (whose parabasal tube has 1–1½ coils) which precedes division. I have found several late division stages where the daughter-parabasals are fully developed and separated, and each has 3–4 coils (Text-fig. 1). This suggests that the two forms described by Janicki are really distinct species, but observations of the division process of slender forms will be necessary to establish this view.



TEXT-FIG. 1.[1]

Devescovina striata Foa, division stage of 'stout form'. Stained with Delafield's haematoxylin. ×412. *ect.*, ectoplasm; *end.*, endoplasm; fl_1. fl_2. fl_3., three anterior flagella; *flt.*, trailing flagellum; *n.*, nucleus; *p.b.*, parabasal organ.

In addition to the two flagellates just mentioned, a new genus and species—Pseudodevescovina uniflagellata is present.

3. From Calotermes oldfieldi var. chryseus Hill.

Staurojoenina assimilis, previously described by Kirby from Calotermes minor, is the largest flagellate, but numbers of Devescovina striata are also found, as well as a species of Oxymonas.

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[1] All figures except 2 B, 5 B and C, and 7 A were drawn with the camera lucida. Magnifications given are approximate, but the actual dimensions are stated in the text.

4. From Coptotermes flavus Hill.

The fauna is similar to that of Coptotermes lacteus Frogg. described by Grassi (1917) except that Holomastigotoides mirabile, previously found in Coptotermes sjostedti replaces Holomastigotoides hemigymnum Grassi.

5. From Coptotermes acinaciformis Hill.

The fauna is identical with that of Coptotermes flavus.

6. From Mastotermes darwiniensis Frogg.

Nyctotherus termitis found by Dobell (1910) in Calotermes militaris Desn. is present.

Also, in this termite there are found species of two new genera which I have named Mixotricha paradoxa and Deltotrichonympha operculata. These will be fully described later.

7. From Stolotermes victoriensis Hill.

A new genus, Spirotrichosoma, is represented by two species, Spirotrichosoma obtusa and Spirotrichosoma capitata. Numerous small Trichomonad flagellates and Tetramitid forms are also present.

SUMMARY OF NEWLY RECORDED PARASITES.

1. From Porotermes grandis Holmgr.
   Trichonympha magna Grassi.
   Joenina pulchella Grassi.
   Spirotrichonympha mirabilis Grassi.
   Spirotrichonymphella pudibunda Grassi.
   Pseudotrypanosoma giganteum Grassi.
   Spirotrichonympha grandis sp. nov.
   Pseudotrypanosoma minimum sp. nov.

2. From Stolotermes victoriensis Hill.
   Spirotrichosoma obtusa gen. et sp. nov.
   Spirotrichosoma capitata gen. et sp. nov.

3. From Calotermes (Neotermes) insularis White.
   Stephanonympha silvestrii Janicki.
   Dev-escovina striata Foa.
   Pseudodevescovina uniflagellata gen. et sp. nov.

4. From Calotermes oldfieldi var. chryseus Hill.
   Staurojoenina assimilis Kirby.
   Devescovina striata Foa.
   Oxymonas sp.

5. From Coptotermes flavus Hill.
   Holomastigotoides mirabile Grassi.
   Pseudotrichonympha hertwigi Grassi.
   Spirotrichonympha flagellata Grassi.

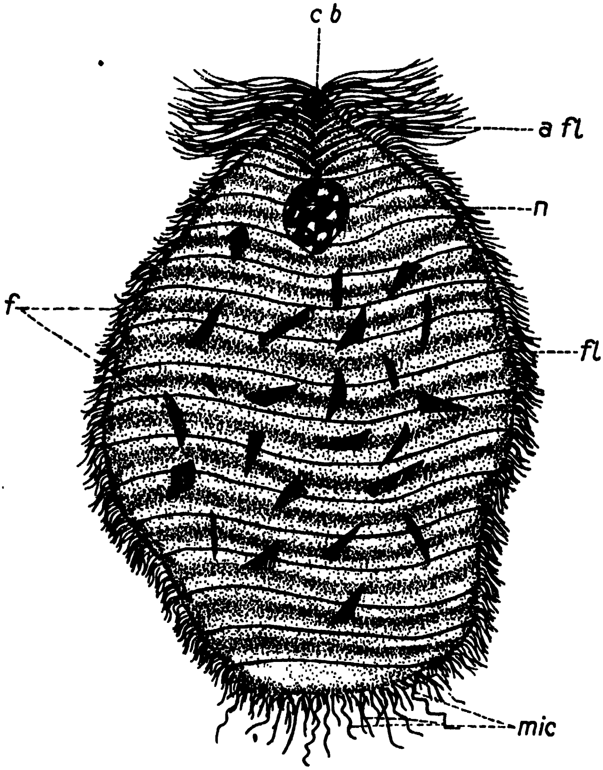
6. From Coptotermes acinaciformis Hill.
   Holomastigotoides mirabile Grassi.
   Pseudotrichonympha hertwigi Grassi.
   Spirotrichonympha flagellata Grassi.

7. From Mastotermes darwiniensis Frogg.
   Nyctotherus termitis Dobell.
   Deltotrichonympha operculata gen. et sp. nov.
   Mixotricha paradoxa gen. et sp. nov.

DESCRIPTIONS OF NEW FORMS.

1. Spirotrichonympha grandis sp. nov. (Text-figs. 2A and B).

This organism shows certain slight but constant differences from Spirotrichonympha mirabilis Grassi, occurring in the same host. Forms intermediate between Spirotrichonympha grandis and Spirotrichonympha mirabilis are never found, and it has been thought advisable to create a new species rather than to describe this flagellate as a variety of Spirotrichonympha mirabilis. The shape is pyriform to oval, the average breadth of the widest part being about 68 μ. The pointed anterior end is actively motile, and the animal swims with a corkscrew-like movement. The average length is about 145 μ but there is a wide range of variation, and forms as long as 200 μ are sometimes found. The nucleus is usually about 11 μ in diameter, and is made up of coarse clumps of chromatin with a well-marked nuclear membrane. It is situated nearer the anterior end than in Spirotrichonympha mirabilis.

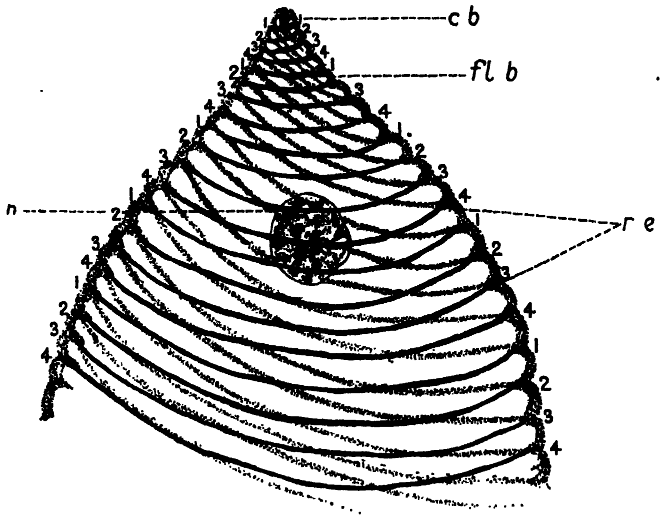


TEXT-FIG. 2A.

Spirotrichonympha grandis sp. nov. Whole individual stained with Delafield's haematoxylin. × 360. Flagella are shown at the edges only. *a.fl.*, anterior flagella; *c.b.*, 'centroblepharoplast'; *f.*, food particles; *fl.*, body flagella; *fl.b.* 1–4, flagellar bands; *mic.*, attached micro-organisms; *n.*, nucleus; *r.e.*, inwardly projecting ectoplasmic ridges which support the flagellar bands.

The pre-nuclear flagellar spiral is closely compressed in front, so that the number of bands is difficult to count. In some stained animals four bands can be traced (Text-fig. 2 B), and it is probable that this number is constant. The spirals are supported by ridges of ectoplasm which project inwards from the body-wall.

There are 3–4 turns of the quadruple spiral investing the body posterior to the nucleus. In surface view they appear as faint, almost transverse, bands. The flagella do not project beyond the posterior end of the body, to which spirochaetes and bacteria are often attached. Those flagella which arise from the thicker portion of the anterior spirals are longer than those covering the body-surface behind the nucleus, and their movement seems to some extent independent of the latter. A short axial organ or 'centroblepharoplast', not so well developed as in other species, can be distinguished at the extreme anterior end. The presence of an axostyle has not been demonstrated



TEXT-FIG. 2B.

Spirotrichonympha grandis sp. nov. Diagram of anterior end to show quadruple spiral of flagellar bands.

For description of lettering, see Text-fig. 2 A.

either in stained or living specimens, although there are sometimes indications of a central clear streak in the protoplasm. By analogy with related forms a broad fibrous axostyle would be expected.

Methods of feeding and division stages have not been observed.

The species Spirotrichonympha grandis may therefore be distinguished from Spirotrichonympha mirabilis Grassi which occurs in the same host by

(*a*) its larger size and oval shape;

(*b*) the arrangement of its flagellar bands, in particular the compression anteriorly of the deeply staining portion of the spiral;

(*c*) the more anterior position of the nucleus.

2. Spirotrichosoma gen. nov.

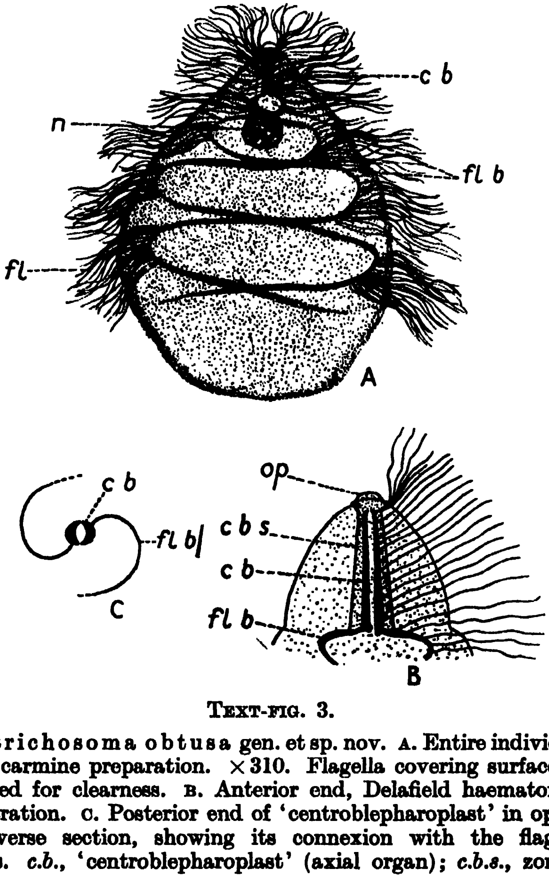
An examination of the alimentary contents of Stolotermes victoriensis shows, in addition to small Tetramitid and Trichomonad forms, numerous hypermastigote flagellates. Among these are two related types for which the genus Spirotrichosoma has been created. That these are distinct species, and not different stages of the life history of one organism is shown (*a*) by constant structural differences, and (*b*) by the occurrence of division stages in both types.

The smaller of the two I have named Spirotrichosoma obtusa, and the larger, Spirotrichosoma capitata.

As will appear from the description of the two species, the genus Spirotrichosoma may be distinguished from the most closely allied genus, Spirotrichonympha by the structure of the axial organ or 'centroblepharoplast' and its relation to the flagellar bands.

3. Spirotrichosoma obtusa sp. nov. (Text-fig. 3).

Length about 62μ. Breadth 36–40μ. The organism is broadly pyriform having a pointed anterior end, but usually no constriction to mark off a head region. The flagellar bands characteristic of the genus, which in this species are very thick cords, are two in number, arranged in a widely spaced double spiral which extends about four-fifths of the length of the body. At the anterior end is an axial organ obviously homologous with the 'centroblepharoplast' of Kofoid, the significance of which is discussed below (see Appendix). This organ consists of two deeply staining rods, usually (Text-fig. 3 A) but not always (Text-fig. 3 B) joined in front and concave in section (Text-fig. 3 C), which are closely apposed to form a tube (Text-figs. 3 B and C).



TEXT-FIG. 3.

Spirotrichosoma obtusa gen. et sp. nov. A. Entire individual, picro-carmine preparation. × 310. Flagella covering surface are omitted for clearness. B. Anterior end, Delafield haematoxylin preparation. C. Posterior end of 'centroblepharoplast' in optical transverse section, showing its connexion with the flagellar bands. *c.b.*, 'centroblepharoplast' (axial organ); *c.b.s.*, zone of clear cytoplasm surrounding the latter; *fl.*, flagella; *fl.b.*, flagellar bands; *n.*, nucleus; *op.*, operculum.

Flagella arise from these rods, which are continuous at their posterior ends with the spiral flagellar bands. Investing the axial organ is a comparatively thick layer of clear protoplasm, and surmounting this at the tip is a small operculum which probably corresponds to the lacuna described by Grassi for Spirotrichonympha. The flagella may be divided into two sets: (*a*) those arising from the sides of the deeply staining rods of the axial organ; (*b*) those arising from the thick spiral bands of the general body surface.

The movement of the head flagella is to a great extent independent of that of the body flagella, and they often form a definite tuft or plume. The nucleus is rounded or oval, and is anteriorly placed, usually immediately behind the 'centroblepharoplast'. When stained with iron haematoxylin, the nucleus shows a coarsely granular structure, and the nuclear membrane is then well defined.

I have seen no trace of an axostyle. Particles of wood are always present in the cytoplasm, but the method of feeding has not been observed.

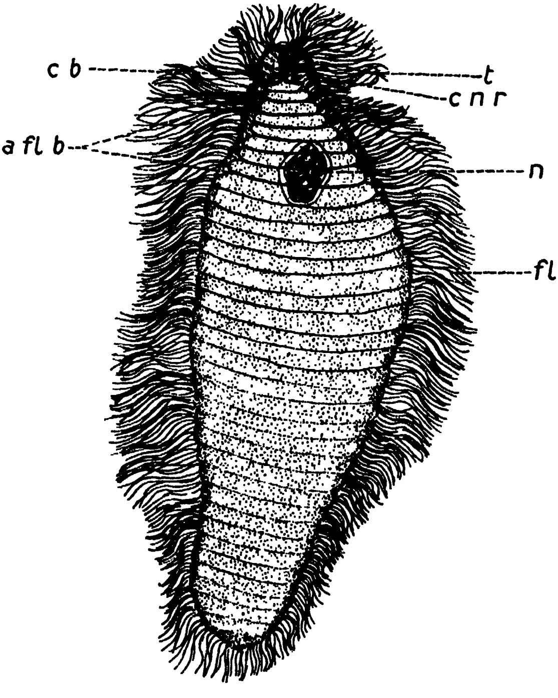
The most obvious distinctive feature of the species Spirotrichosoma obtusa lies in the arrangement and structure of the flagellar bands. These are very coarse, and are disposed in a widely spaced double spiral of about one and a half turns.

4. Spirotrichosoma capitata sp. nov. (Text-fig. 4).

Length about 97 μ. Breadth 38 μ at the widest point.

The organism is elongated, tapering at each end. A definite constriction is usually present at the level of the posterior end of the 'centroblepharoplast', so that a head region can be distinguished from the rest of the body.

The flagellar bands, which again are two in number, can best be counted in distorted specimens where the anterior end has been pulled out. The bands which are very much thicker in the anterior quarter of the body are very closely interwoven to form a fine tight spiral, which becomes extremely faint towards the posterior end. In front they end in conjunction with the 'centroblepharoplast' which has the same structure as that described for Spirotrichosoma obtusa. The anterior tuft of flagella is very well developed, and is composed both of the flagella arising from the sides of the 'centroblepharoplast', and of those from the most anterior turns of the spiral at the base of the head region. This arrangement is probably correlated with the closeness of the spiral twisting, which brings the anterior turns nearer to the 'centroblepharoplast' than in Spirotrichosoma obtusa.



TEXT-FIG. 4.

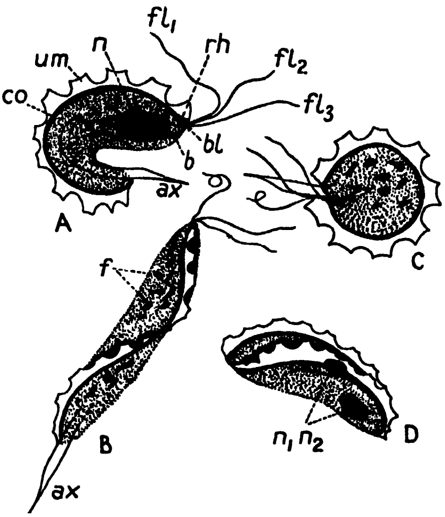
Spirotrichosoma capitata gen. et sp. nov. *a.fl.b.*, thickened anterior portion of flagellar bands; *c.b.*, 'centroblepharoplast' (axial organ); *c.n.r.*, constricted neck region; *fl.*, flagella; *n.*, nucleus; *t.*, anterior tuft of flagella.

The nucleus shows the same structure as in Spirotrichosoma obtusa, but is often at a greater distance behind the 'centroblepharoplast'.

Spirotrichosoma capitata may therefore be distinguished from Spirotrichosoma obtusa by: (*a*) the much closer winding, and therefore more numerous turns, of the spiral flagellar bands; (*b*) the sharper demarcation of the head region.

5. Pseudotrypanosoma minimum sp.nov. (Text-fig. 5).

Kirby (1931) recorded 'a small Trichomonad flagellate' from

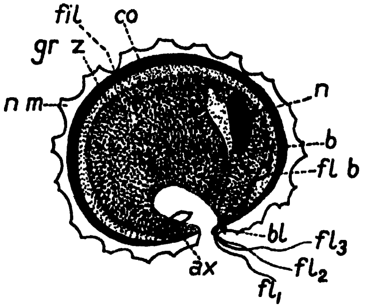


TEXT-FIG. 5.

Pseudotrypanosoma minimum sp. nov. A. From a Delafield haematoxylin preparation. ×412. B and C. Living forms to show changes of shape. D. Division stage. *ax.*, axostyle; *b.*, band from nucleus; *bl.*, blepharoplast; *co.*, costa; *f.*, ingested food; fl_1. fl_2. fl_3., flagella; *n.*, nucleus; n_1. n_2., daughter nuclei; *rh.*, rhizoplast; *u.m.*, undulating membrane.

Porotermes grandis Holmgr., but owing to insufficiency of material was unable to decide whether or not it represented a new species. An organism, apparently identical with that mentioned by Kirby has been constantly present in my material. Evidence that this form is not a young stage in the life history of Pseudotrypanosoma giganteum Grassi (Text-fig. 6), which is found in the same host, is supplied by the occurrence of division stages, which have been observed both in living material and in stained smears. Also, although large numbers of termites have been examined, no transitional stages between the two forms have been found.

The average length is about 33–7 μ and breadth about 11 μ. As in Pseudotrypanosoma giganteum (Text-fig. 6) the body assumes various shapes when alive, but the general type of movement is different in the two species. Pseudotrypanosoma giganteum has a tendency to round itself into an arc, having only the flagella and the undulating membrane active and varies this occasionally with a sudden stretching and twisting movement.



Text-fig. 6.

Pseudotrypanosoma giganteum Grassi. Stained with Delafield's haematoxylin. ×412. *ax.*, axostyle; *b.*, band from nucleus; *bl.*, blepharoplast; *fil.*, filament; fl_1. fl_2. fl_3., anterior flagella; *fl b.*, backwardly directed flagellum; *gr.z.*, granular zone; *n.*, nucleus; *u.m.*, undulating membrane.

Pseudotrypanosoma minimum, on the other hand, is constantly turning in a jerky fashion reminiscent of that described by Cutler (1919) for Ditrichomonas. As Kirby has described, there are three fine anterior flagella, relatively longer than in Pseudotrypanosoma giganteum. The undulating membrane has typically fewer and larger undulations in proportion to its length than in Pseudotrypanosoma giganteum and the costa or supporting base is comparatively slender.

A fairly large blepharoplast from which the flagella arise is present at the anterior tip. The nucleus, which is situated anteriorly is relatively very large, and oval or kidney-shaped. In carefully differentiated iron haematoxylin preparations it is shown to be vesicular, but with other stains it appears homogeneous.

In spite of abundant material further details of the structure of the organism have proved exceedingly difficult to investigate, and have not in all cases been determined with certainty.

A well-developed axostyle is present, passing back from the region of the nucleus to end in a lance-shaped tip which projects from the hind end. The anterior end of the axostyle is only dimly visible. It seems to vary in position; it may be in front and above, just behind, or a considerable distance behind the nucleus.

There appear to be no structures corresponding to the filament on the granular zone or the occasional backwardly directed flagellum of Pseudotrypanosoma giganteum regarded by Kirby as a developing costa. A fine thread-like rhizoplast has occasionally been seen connecting nucleus and blepharoplast (Text-fig. 5 A).

There occurs also at the anterior end, close to the nucleus, and usually obscured by it, a peculiar organ which evidently corresponds to the 'band to the nucleus' (Kirby) of Pseudotrypanosoma giganteum. Its nature must at present remain uncertain. Even in Pseudotrypanosoma giganteum, where it is much more evident, its structure has hitherto not been elucidated. Careful examination of this species (Pseudotrypanosoma giganteum) in my material seems to show that the undulating membrane is continued round the anterior tip of the organism as two ridges that run back on its under surface, enter the protoplasm as a tube beneath the nucleus, and expand there to form an irregular chamber. Its construction suggests a possible cytopharynx, although there is no direct evidence on the living organism to support such a view.

The division stages observed were all advanced, the nucleus and undulating membrane having split, although the daughter-individuals had not separated; the mitotic processes cannot therefore be described.

The species Pseudotrypanosoma minimum, therefore, differs from Pseudotrypanosoma giganteum in its smaller size, in its method of locomotion and movement, and in the characteristic undulations of the membrane. It also differs in the larger relative size of the nucleus, which is placed nearer the anterior end, and much nearer the under surface than in Pseudotrypanosoma giganteum.

6. Pseudodevescovina uniflagellata gen. et sp. nov. (Text-fig. 7).

These organisms, although comparatively large and constant in occurrence, are much less numerous than the other inhabitants of the gut of Calotermes (Neotermes) insularis White. The length is about 65 μ, while the average breadth is 40–45 μ.

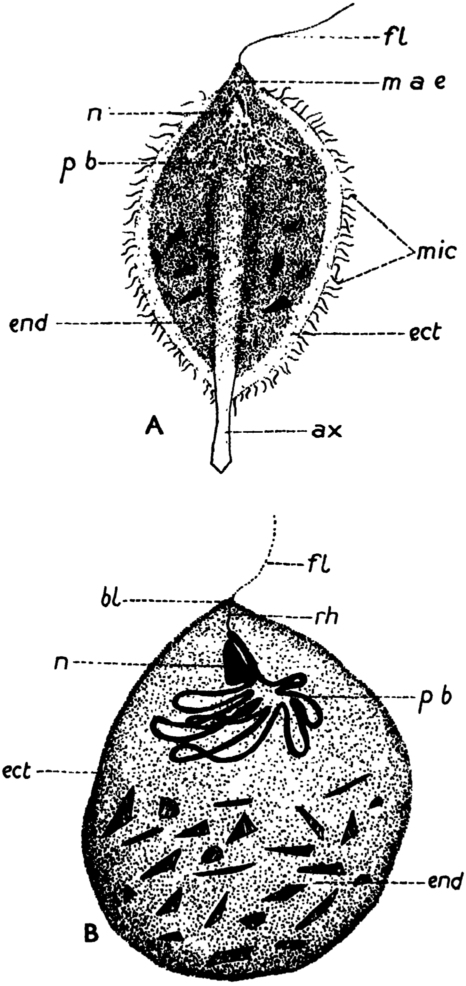
The shape of the living animal is oval or pyriform, tapering either at the anterior end only, or at both ends. It usually becomes rounded off and shapeless after a few minutes in saline, although various concentrations have been tried.

The pointed anterior end, which is actively motile, bears a single flagellum arising from a blepharoplast. There is no trailing flagellum. The protoplasm is differentiated into an outer, clear, cortical ectoplasm and an inner, granular endoplasm containing large food-particles; this differentiation is especially well shown in some stained specimens, where the endoplasm tends to shrink away from the ectoplasm.

As in Devescovina, it is probable that food ingestion may occur at any point of the general body-surface, and large particles of wood are present in the endoplasm.

The nucleus, which resembles that of Devescovina, is coarsely granular and roughly conical.

The parabasal body consists of a much-looped, darkly staining strand. The number of loops seems to be variable, and no definite spiral is formed. An anterior limb of the parabasal passes forward to the front of the nucleus as in Devescovina, and a fine thread, the rhizoplast, connects the parabasal with the blepharoplast.



TEXT-FIG. 7.

Pseudodevescovina uniflagellata gen. et sp. nov. A. Living form. B. Stained with Heidenhain's iron haematoxylin. ×600—the axostyle is withdrawn. *ax.*, axostyle; *bl.*, blepharoplast; *ect.*, ectoplasm; *end.*, endoplasm; *fl.*, flagellum; *m.a.e.*, motile anterior end; *mic.*, attached micro-organisms; *n.*, nucleus; *p.b.*, parabasal organ; *rh.*, rhizoplast.

The axostyle, which ends anteriorly in the region of the parabasal, is well developed, but more easily visible in living than in stained forms.

As in Devescovina stereociliata, the body-surface is usually coated with micro-organisms, superficially like cilia and often actively motile with a rapid flickering motion different from the co-ordinated movements of true cilia.

Systematic Position.—Probably, together with Mixotricha paradoxa from Mastotermes darwiniensis, this form is an aberrant Tetramitid. It resembles Devescovina in general appearance, in the shape and position of the nucleus, in the presence of a complex parabasal apparatus and axostyle, and in the structure of the anterior end.

The main differences between this genus and Devescovina are: (*a*) the reduction of the anterior flagella to one; (*b*) the absence of a trailing flagellum; (*c*) the arrangement of the parabasal apparatus. It also shows resemblance to Mixotricha paradoxa in general plan and in the presence of a motile anterior tip, but differs in the absence of the investing coat of cilia, and also in the further reduction in number of the anterior flagella. The looped parabasal strand of Pseudodevescovina may perhaps be regarded as homologous with the straight strand connecting nucleus with blepharoplast in Mixotricha, although the relative positions of nucleus and parabasal are different in the two forms.

The protozoa from Mastotermes darwiniensis are extremely interesting. This termite is very primitive, and is described by Desneux (1904) as resembling the Blattidae in wing-venation, tarsal formula, and the structure of the antennae.

Although Mastotermes can live on various kinds of wood, they are most plentiful in the sugar-cane fields of North Queensland. The intestinal contents are usually very liquid.

A ciliate conforming to Dobell's description of Nyctotherus termitis from Calotermes militaris is present in the alimentary canal. The large flagellate parasites are so unusual as to warrant the creation of two new genera.

7. Mixotricha paradoxa gen. et sp. nov. (Text-fig. 8 A, B, C, D).

The largest flagellate of Mastotermes darwiniensis, this form is clearly visible to the naked eye. The organism is about 340 μ in length, 200 μ in breadth, and 25 μ in thickness, i.e. it is extremely flattened.

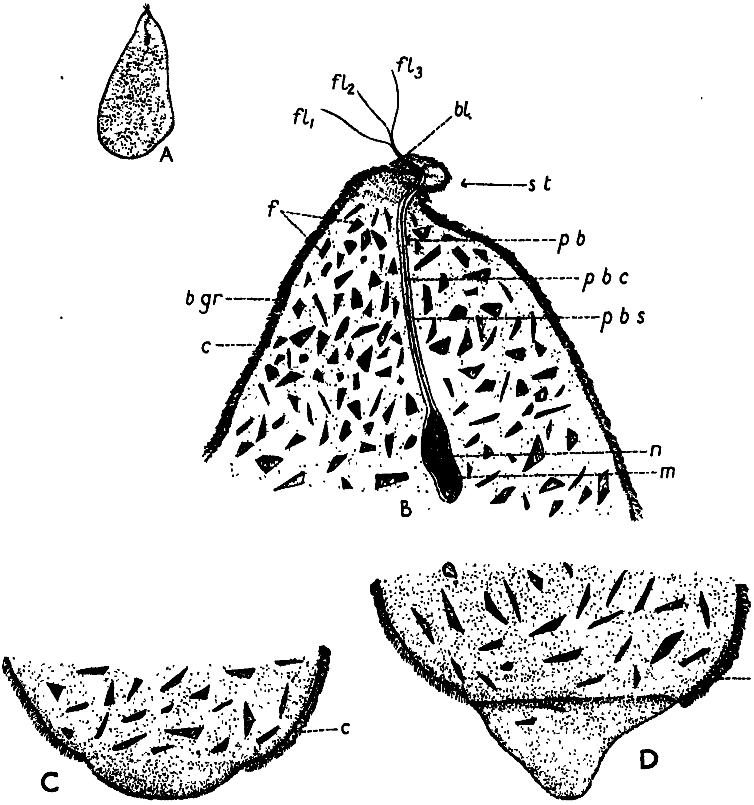
The shape is shown by Text-fig. 8 A and is usually fairly constant, but the body may occasionally be thrown into folds. The anterior tip has a characteristic spiral twist, and is very motile. The extreme posterior region, which is devoid of cilia, seems to be eversible, and often shows a protruding plug of granular protoplasm. The body is bounded by a definite ectoplasmic cortical zone, which is very thick at the front end where it composes the anterior tip, but becomes thin over the posterior protuberance.

The body is invested with a coat of cilia disposed in closely packed transverse bands. Their insertion and movement are quite different from those of the short flagella of Trichonymphids, and they can definitely be distinguished from the foreign organisms which invest such forms as Pseudodevescovina by the nature of their movement, and by their basal granules situated in the cortical zone, which show clearly in stained smears, and especially in distorted specimens where the ectoplasm has shrunk away from the endoplasm.

The pointed anterior end bears three, fine, relatively short flagella arising from three close-set blepharoplasts. The flagella are hard to demonstrate clearly in stained preparations owing to their tendency to tangle, but they are plainly visible in the living animal.

The nucleus is relatively very small, being about 20 μ long by 7 μ broad. Surrounding the nucleus is a narrow clear zone, invested by a membrane which is prolonged in front of the nucleus as a narrow tube extending as far forward as the blepharoplasts. In carefully differentiated iron haematoxylin preparations, a thread-like core appears along the length of the tube—ending behind at the anterior tip of the nucleus. It is possible that the tube represents a parabasal structure.

The blepharoplasts are three granules, staining well with



TEXT-FIG. 8.

Mixotricha paradoxa gen. et sp. nov. A. External features. B. Anterior portion stained with Heidenhain's iron haematoxylin. Anterior tip is in surface view to show spiral twist, the remainder is in optical section. × 412. C. Protrusible posterior end in normal position. D. The same, protruded. *b.gr.*, basal granules of cilia in cortical zone; *bl.*, three blepharoplasts; *c.*, cilia; *f.*, ingested food particles; $fl_1 . fl_2 . fl_3$., three anterior flagella; *m.*, membrane investing nucleus; *n.*, nucleus; *p.b.*, tube from the nucleus of the blepharoplasts; *p.b.c.*, deeply staining core of tube; *p.b.s.*, outer sheath of tube; *s.t.*, spiral twist.

iron haematoxylin, but usually difficult to distinguish individually.

The endoplasm is filled with wood particles, but they are absent from the granular protoplasm of the extreme hind end. Feeding probably occurs by invagination at the hind end (Text-fig. 8 C, D) as described by Cleveland for Trichonympha campanula, but the actual process has not been observed. There is no cytopharynx.

In general appearance, and particularly in the presence of both cilia and flagella, this form is quite unlike any described termite parasite. It is also worthy of note that Mixotricha occurs in the most primitive, known termite. The presence of cilia as locomotor organs may be correlated with the liquid surroundings, where there is no need to push aside large particles by vigorous movements.

Except for the presence of cilia, this form has no diagnostic characters in common with the Infusoria. There is only one nucleus, and the blepharoplasts and anterior flagella are all typical of Mastigophora.

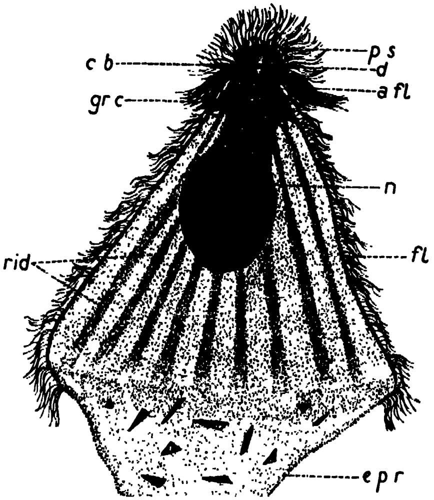
Rather than make a new family to contain Mixotricha, it has been placed, provisionally, as an aberrant Tetramitid, since, apart from its cilia, it has some characters in common with the Devescovinids.

The simple anterior tube of Mixotricha paradoxa may be homologous with the spiral body of Devescovina and the much-looped strand of Pseudodevescovina.

The fine anterior flagella attached to an actively motile tip are also suggestive of Devescovina, although, as in Pseudodevescovina the trailing flagellum is absent.

8. Deltotrichonympha operculata gen. et sp. nov. (Text-figs. 9A, B, and C).

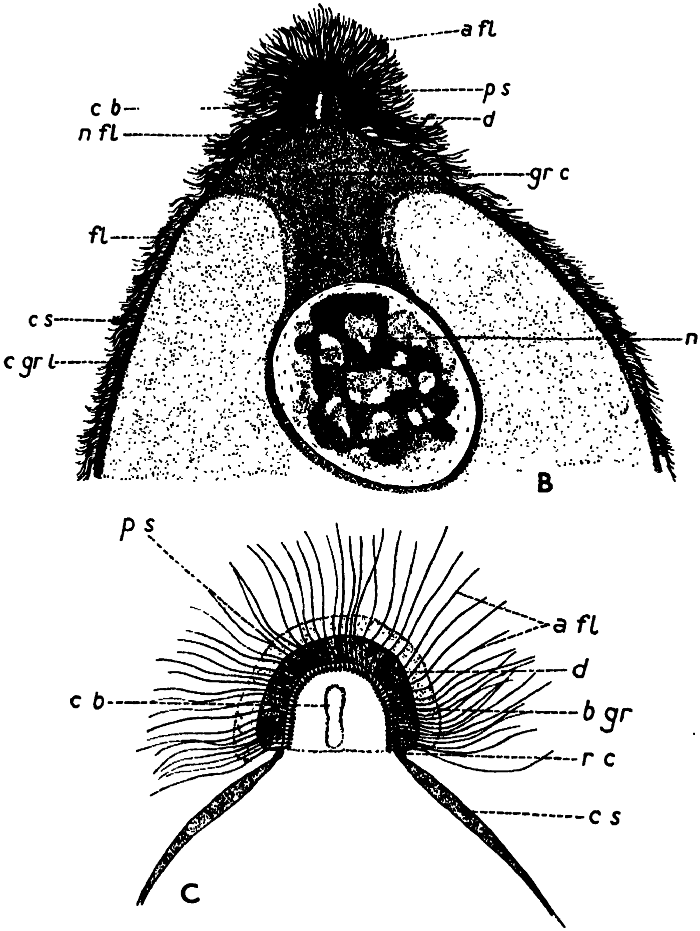
This organism is easily visible to the naked eye. The length from the anterior tip to the beginning of the naked eversible posterior region is about 180μ, when fully expanded it may reach 230μ; the breadth at the widest part is about 164μ. The organism has a thickness of about 50μ, and therefore exhibits bilateral symmetry. It may be described as roughly triangular, the apex of the triangle being a small dome-shaped 'head'. At the hind end is a soft protuberance; this is the organ which is presumably used for the intake of food. The 'head' has a dense coating of long, actively motile flagella, those forming a ring around the 'neck' region being particularly well developed. Over the rest of the body the flagella are shorter and are arranged in five longitudinal rows which radiate outwards and backwards from the base of the head, and resemble those of Pseudotrichonympha. The large protuberance at the hinder end is devoid of flagella, and is usually covered with attached micro-organisms.



TEXT-FIG. 9A.

Deltotrichonympha operculata gen. et sp. nov. Entire animal, from a picro-carmine preparation. × 180.

Supporting the flagella over the surface of the animal is a thin cortical sheath. This gradually thickens in front, and is joined by an apparently flexible thinner ring to a thick-walled dome in the head region. Probably it is the flexibility of the connecting part which allows of the free play of the 'head' seen in the living animal. The dome-like, thickened sheath in the 'head' is evidently correlated with the presence in this region of the more powerful flagella. The sheath is absent from the posterior protuberance.



TEXT-FIG. 9A and B.

Deltotrichonympha operculata gen. et sp. nov. B. Anterior end to show nuclear support, outer cortical sheath, and origin of the anterior flagella. C. Diagram of anterior end. *a.fl.*, anterior flagella; *b.gr.*, basal granules; *c.b.*, centroblepharoplast (?); *c.gr.l.*, cortical granular layer; *c.s.*, outer sheath of cortex; *d.*, dome; *e.p.r.*, eversible posterior region; *fl.*, flagella; *gr.c.*, anterior concentration of granular cytoplasm; *n.*, nucleus; *n.c.*, thinner sheath of 'neck' region which forms a flexible hinge between dome and body; *n.fl.*, well-developed ring of flagella in neck region; *p.s.*, protoplasmic sheath; *rid.*, ridges of body-wall.

The examination of the mode of insertion of the anterior flagella presents some difficulty. In many preparations, particularly specimens fixed in osmic acid and stained with picrocarmine, the domed supporting structure in the head seems to be invested with a comparatively thick sheath of protoplasm from which the flagella emerge. But in other individuals, especially in iron haematoxylin material, the sheath may be resolved into small rods which seem to be the proximal thickened ends of the flagella. Whether this appearance is due to fracturing of the protoplasmic sheath which is continuous in the living animal, I have not been able to determine, and living material has not proved helpful.

In iron haematoxylin preparations it is possible to see that the flagella of the head go through the supporting structure and are connected with minute basal granules that form a layer below it (Text-fig. 9 c).

A small axial rod-shaped body, found in the centre of the head region, and showing best in iron haematoxylin preparations, may be a centroblepharoplast, but in no case is its outline as clear as in other Trichonymphids.

The cytoplasm of the organism may be divided into regions: (*a*) an outer granular, and (*b*) an inner more fluid. The granular portion fills the anterior part of the body for a short distance behind the head, and is continued as a thin outer layer over the entire animal. It also dips down as a central core into the more fluid portion and forms a support for the nucleus.

The more fluid inner cytoplasm fills the rest of the body and constitutes the whole of the posterior protuberance.

The nucleus is large, about 57 μ in length by 30 μ in breadth, and is placed in the anterior third of the body. The chromatin is in the form of a coarse network, and the nuclear membrane is well developed.

Numerous wood fibres are present in the body, and food intake presumably occurs by invagination of the posterior fleshy protuberance.

The distribution of flagella is like that found in Pseudotrichonympha, except for their different arrangement at the highly modified anterior end. The nuclear support and eversible posterior end suggest the condition found in Trichonympha. The dome-shaped head region may be compared with the typical Trichonymphid nipple as described by Koidzumi. The probable nature of the 'head' structures is more fully discussed in the appendix.

The rounded anterior tip at first sight seems to show relationship with Gymnonympha zeylanica Dobell, but in this latter form the relation of cap to flagella is entirely different. It has therefore been thought best to place Deltotrichonympha as a somewhat aberrant member of the Trichonymphidae, having slight affinities with Trichonympha and Pseudotrichonympha.

APPENDIX.

Nature and Significance of the Axial Organ or 'Centroblepharoplast'.

Kofoid and Swezy, working on Trichonympha campanula, described an anterior axial organ made up of deeply staining filaments with a clear central core. This organ they named centroblepharoplast, assigning to it both a neuromotor function as a co-ordinating centre for the flagellar complex and also a centrosomic function in mitosis.

Its blepharoplastic function is deduced from its morphological relation to the flagellar bands, and its centrosomic function is evidenced by the fact that the organ divides before the nucleus, and forms a spindle or paradesmose which controls the distribution of the divided chromatin.

Bĕlăr accepts the view that at the base of the 'rostrum' (centroblepharoplast of Kofoid and Swezy) in Trichonympha there is a centrosome-like structure giving rise at mitosis to a centro- or para-desmose. This structure is however no longer visible as a morphological element, but manifests itself at mitosis as a zone between the separating portions of the 'centroblepharoplast'. He therefore considers it wrong to apply this name to the whole of the organ in question.

A few telophase division stages occur in my material of Spirotrichosoma, and these support Bĕlăr's doubts as to the propriety of assigning a centrosomic function to the organ itself. The axial organ (centroblepharoplast) is in the form of an inverted V, being still undivided at the apex, while at its base the two components are widely divergent in the direction of the long axis of the greatly elongated nucleus. This lies well below the level of the ends of the two arms of the axial organ, and moreover the telophase masses are far more widely separated than the maximum divergence of the arms. The whole picture is thus very different from that depicted by Kofoid and Swezy in Trichonympha.

In Spirotrichosoma, therefore, it does not seem possible that the axial organ plays the part in mitosis ascribed to it in Trichonympha by Kofoid and Swezy, and if it has any relation to a centrosomic function it can scarcely be more than a topographical relation to a centrosomic zone as suggested by Bělăr. On the other hand, there is evidence that the organ has a skeletal function. In my material, cases have been found in which the animals have become distorted, and even broken in fragments, but in which the axial organ and the flagellar bands have survived. It is evident that they are of a much tougher consistency than the rest of the animal, and therefore may well serve as supporting structures, doubtless for the flagella that arise from them. The increased thickness of the supporting organ in the 'head' is perhaps to be correlated with the greater length of the flagella here. A skeletal function for the axial organ has already been suggested by Grassi and others.

These conclusions are borne out by observations on Deltotrichonympha. There occurs here a dome-shaped organ within the head, which is evidently very similar to the axial organ of Spirotrichosoma. It seems to act as a support for the large flagella which arise from the head—a thinner sheath sufficing for the shorter flagella that cover the rest of the body. A blepharoplastic function is even less likely than in Spirotrichosoma, for in Deltotrichonympha the flagella can be seen to arise from a layer of basal granules on its inner wall (Text-fig. 9 c). There also occurs within the head a rod-like body which I have suggested may be a true centroblepharoplast—but in the absence of direct observation of its behaviour in division its homology must for the present remain undetermined.

From these observations it seems clear that, whatever may be the function of this peculiar structure in Trichonympha campanula, it does not function as a centrosome nor even apparently as a blepharoplast in other Trichonymphids.

SUMMARY.

1. A list is given of protozoa previously described from termites of different countries, and now recorded from Australian termites.

2. Observations on division stages of Janicki's 'stout form' of Devescovina striata do not support his contention that the 'stout form' is a pre-division stage of the 'slender form'.

3. A new species Spirotrichonympha grandis is described from Porotermes grandis Holmgr.

4. The protozoa of Stolotermes victoriensis Hill include a new genus, Spirotrichosoma, of which two species occur—Spirotrichosoma obtusa and Spirotrichosoma capitata.

5. A Trichomonad flagellate from Porotermes grandis, mentioned but not described by Kirby, has distinctive characters which mark it as a new species, Pseudotrypanosoma minimum.

6. A new genus Pseudodevescovina (Pseudodevescovina uniflagellata) is described from Calotermes insularis White.

7. Mastotermes darwiniensis Frogg., the most primitive known termite, contains in addition to a ciliate, Nyctotherus termitis, large and unusual flagellates for which the genera Mixotricha (Mixotricha paradoxa) and Deltotrichonympha (Deltotrichonympha operculata) have been erected. The morphology and possible affinities of these forms are of great interest.

8. Observations made on the 'centroblepharoplast' of Spirotrichosoma obtusa tend to show that this organ is in this form primarily skeletal rather than neuromotor in

function. Division stages show that in Spirotrichosoma obtusa the axial organ does not function as a centrosome, so that the term 'centroblepharoplast' is inexact for this form. An analogous skeletal or supporting structure is found in Deltotrichonympha.

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On the Anatomy of some Fish Cestodes described by Diesing from the Amazon.

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With Plates 11 to 16 and 1 Text-figure.

In 1850 and 1855 Diesing briefly described the superficial features of about half a dozen Cestodes obtained by Natterer from fishes in the Amazon and on this basis he founded five genera. Zoologists have been familiar for many years with Diesing's figures of the remarkable scoleces of some of these Cestodes (as reproduced in Braun's account of the Cestodes in Bronn's 'Klassen und Ordnungen des Thier-Reichs', e.g.) but apparently, with the sole exceptions of Ephedrocephalus microcephalus redescribed in 1906 by Mola, and of the Cestodarian Amphilina liguloidea (the Monostomum liguloideum of Diesing and the Schizochoerus liguloideus of Poche), no one has, up to the present, had the opportunity of ascertaining the strobilar anatomy corresponding to these scoleces. In fact fish Cestodes from the Amazon, apart from the descriptions mentioned and the papers by Fuhrmann (1916) on Goezeella siluri from Cetopsis caecutiens, and by Janicki (1908) on Amphilina (Nesolecithus) janickii Poche (described under the name Amphilina liguloidea—a Cestodarian; see Poche 1925, p. 257), are, so far as I am aware, quite unknown, though three or four species have been described from other parts of South America. This being the case, the outline description of the strobilar structure of six of Diesing's species of Cestodes contained in this paper may be of more than usual interest.

The material on which the following descriptions are based was obtained by me during the latter half of 1931. In that year

I was able, thanks to the generosity of Sir Henry Wellcome, F.R.S., and the kind offices of Dr. C. M. Wenyon, F.R.S., to traverse the main channels and backwaters of more than 1,100 miles of the Rio Amazonas and Rio Solimões (Alto Amazonas) for the purpose of collecting Helminth parasites. Of fishes I examined altogether 312 individuals belonging to sixty-five distinct species, and the majority of these were caught on hook and line or in circular hand-nets by the crew of the two boats which I purchased for the voyage back to Pará from Manaós. I had at first hoped to be able to utilize the river steamers as a means of transport and to engage local fishermen to obtain fishes and other animals for my purposes, but a pr liminary visit to a typical village (Codajaz, about 150 mile above Manaós) soon convinced me that native indolence was proof against all practicable forms of inducement. The fish market at Manaós was the only other source of supply available to me, but this of course only represented one locality, and non-edible fish were rarely to be seen. I had also relied greatly upon the fish markets at Obydos, Santarem, and other small towns or villages and especially at Pará, but I found that all markets other than the large Portuguese-managed concerns at Manaós and Pará were very poorly supplied and quite useless to me, and I was prevented from profiting by the excellent market at Pará by becoming incapacitated with acute kidney trouble towards the end of the voyage, and the last fish I examined was up a creek at Gurupa some 200 miles from Pará.

The parasites I sought for were mainly Cestodes, and of these I obtained a large number from fishes and birds, those from fishes mostly new to science. Unfortunately, in the cases of five out of the six species described by Diesing, the material I obtained was very scanty, though sufficient to describe the main features of the anatomy. Only in the case of Amphoteromorphus peniculus was my material plentiful, though my description of this species is not correspondingly amplified.

Finally, I wish to acknowledge my obligations to the following gentlemen for kind assistance in connexion with my visit to Brazil: Dr. L. W. G. Malcolm of the Wellcome Historical Medical Museum, Mr. W. H. Parker, Professor R. Ruggles Gates, His

Excellency Senhor Raul Regis de Oliveira of the Brazilian Embassy, His Excellency Senhor Emmanuel Morães, Acting-Governor of the State of Amazonas at the time of my visit, and Mr. F. G. Coultas, British Consul at Pará. I also wish to thank Messrs. J. R. Norman and N. B. Kinnear of the British Museum for identification of the fishes and birds which I examined.

Zygobothrium megacephalum Diesing, 1850.
(Figs. 1–17, Pls. 11 and 12).

This tapeworm is found in the middle intestine of the Pirararà, the Pirarara bicolor of Spix and Agassiz, and the Phractocephalus hemiliopterus of Cuvier and Valenciennes, a common and ferocious fish and very conspicuous with its dark grey dorsum, yellow lower surface, and sides with black patches, and brilliant scarlet fins. My largest examples were about 1 metre in length—not far short of the maximum length recorded, viz. 1·2 metres. Out of 27 of these fishes examined, 6, captured between Manaós and Santarem, contained Zygobothrium megacephalum, and of this species I only possess 5 entire mature specimens, 2 large scoleces (with portions of strobila), and 2 small immature specimens.

External Features.

Diesing gives the maximum length of the entire worm (fig. 1, Pl. 11) as 3 in., which I assume to be 76 mm., and the maximum breadth as 2½‴ or about 5 mm. My largest specimen measured 70 mm. in length and 3 mm. in maximum breadth (preserved in formalin). The scolex is, as the specific name implies, large, being, in my largest specimen, 3 mm. broad and 2 mm. high (these measurements were doubled in Diesing's largest specimen), and essentially consists of four large hollow globular parts united centrally to a central column and also fused to each other laterally (fig. 2, Pl. 11). Each hollow globular part is in reality a large sucker which, however, differs from most other suckers in having its wide opening to the exterior divided into two by a relatively narrow though conspicuous bridge of tissue disposed tangentially to the scolex circumference (figs. 2–5, Pl. 11)—a feature characteristic of this species. The figures of

Diesing show the scolex in an unattached and somewhat contracted condition, the two openings of each sucker[1] being very small and the surface very wrinkled. Fig. 2, Pl. 11, shows the scolex less contracted, and fig. 5, Pl. 11, is a vertical section through another scolex in the same condition. Fig. 4, Pl. 11, shows an end-on view of the scolex in a contracted condition similar to that figured by Diesing. The central area inside the sucker openings is sometimes slightly elevated. There is no 'os terminale' (Diesing queried its existence) nor apical organ of any kind. The axis of the central column of the scolex contains numerous muscle-fibres, and each globular wall of the sucker is lined with a layer of muscle specially thickened round the edge of the opening. Fig. 3, Pl. 11, shows the end-on appearance of a scolex which was attached to the mucosa by four large digitiform masses of mucosa which projected in through the inner openings and emerged as lumps through the outer openings of each sucker. In fig. 7, Pl. 11, in which all eight openings of the suckers are in contact with the mucosa, it is possible that the inner and outer openings of the suckers acted each as a separate sucker. As this figure shows, the scolex can become greatly deformed during attachment.

An unsegmented neck region is absent. In the strobila the segments are very numerous, are very short anteriorly, and only attain a length equal to or exceeding their breadth at the extreme posterior end. Mature segments (in which all the organs are well formed, but eggs have not entered the uterus) first occur about midway in the length of the strobila. The most conspicuous feature about the strobila is the great growth of the posterior borders of all the segments (figs. 8–10, Pl. 11), so that, both dorsally and ventrally, the actual strobila is overlaid by at least two thicknesses of border lappet and, unless the lappets are scraped off, it is useless to prepare a piece of strobila as a whole-mount. As Diesing remarks, each surface of the strobila shows a median furrow, marked by median notches in the lappets. On the ventral side the uterus openings coincide

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[1] I use the term sucker because its cavity has a distinct though thin muscle-layer clearly delimited from the underlying parenchyma; a bothrium has no musculature apart from that traversing the general parenchyma.

with the median furrow. The cirrus and vaginal apertures are marginal, opening at about the middle of the length of the segment, and very irregularly alternate (as many as nine cirrus sacs, e.g. may occur consecutively on the same side). In very few of my specimens are the cirri protruded prominently, and in these cases atria are absent; in other cases the retracted cirrus and vagina open into a shallow (fig. 17, Pl. 12) or relatively deep atrium and therefore possess a single opening on the proglottid surface; in other words, the atrium is largely a temporary structure, present chiefly in immature proglottids. The openings of the cirrus and vagina in young proglottids are covered over by the lappets of the proglottid in front but become uncovered as the segments become mature and elongate.

Internal Anatomy of the Proglottid.

I first will describe briefly the structure of a mature proglottid. The testes are numerous (between 150 and 200 in number on a rough estimate) and are scattered more or less uniformly in the medullary parenchyma (i.e. inside the longitudinal muscle-layer) over the dorsal surface of the proglottid between the lateral groups of vitellaria, except in the area occupied by the branching ovary along the posterior border (fig. 11, Pl. 11). In balsam whole-preparations the testes measure approximately 66 microns in diameter. The cirrus sac is about 0·66 mm. long and 0·083 mm. broad, and it opens ventrally and posteriorly to the vagina, usually in a shallow atrium (fig. 17, Pl. 12), but in immature segments the atrium is much deeper and more sharply defined. The wall of the sac is thin, hyaline, and quite devoid of muscle-fibres and, with the cirrus retracted, contains (*a*) a straight canal, thick-walled (with ejector muscles attached), and with lumen lined with chitinous spines, which occupies the outer two-thirds of the sac and forms the eversible spiny cirrus, and (*b*) the inner convoluted ductus. The coils of the vas deferens are in young mature segments very thin and convoluted, running ventrally to the vagina, but, in older proglottids, the vas dilates enormously (fig. 15, Pl. 12) with accumulated masses of sperms (vesicula seminalis). The vagina opening is contiguous with, and dorsal to, that of the cirrus sac, and the dilated outer end of the vagina may lie anterior or posterior to the cirrus sac, though the main vagina canal, in all the preparations I have examined, always lies posterior (and dorsal) to the vesicula seminalis. At its outer end the vagina possesses a muscular thickening in its wall, the so-called sphincter vaginae. The vagina pursues a straight course to the ventral side of the median part (isthmus) of the ovary, but I am unable to elucidate the detailed composition of the genital complex. The ovary, while immature, consists of a large number of follicles borne on a system of branching tubes which occupies the posterior border of the proglottid on its dorsal side (fig. 11, Pl. 11). In a more mature condition the ovary follicles lose their distinctness owing to individual enlargement. The uterus arises as a narrow outgrowth from the central ovary complex on the ventral side and, while extending forwards in the median line, gives off lateral diverticula similar to those found in a species of Taenia (figs. 11, 12, Pl. 11).

The vitellaria are small ovoid bodies (*c.* 25·5×14·5 microns) arranged in two small semicircles, each semicircle embracing the lateral bend of the circular band of longitudinal muscles. This arrangement is similar to that found in a typical Phyllobothriid save that in this family the vitellaria lie internal and not external to the longitudinal muscle-bundle system. There are also, in Zygobothrium megacephalum, a few vitellaria present in the middle line on the dorsal side, also external to the muscle-band (figs. 14, 16, Pl. 12). This is reminiscent of a primitive condition in which, as in many existing Tetrarhynchidae, the vitellaria are disposed in a complete circle in transverse sections of the proglottids.

The circular band of longitudinal muscles delimiting the cortex from the medulla (figs. 14–16, Pl. 12), is composed of the usual bundles of fibres, but in the young and mature proglottids these bundles are so compacted together that they can only be distinguished under the higher powers of the microscope; only in gravid proglottids, when the muscle-system is degenerating, do the individual bundles become mutually separate and therefore distinct. I cannot detect transverse muscle-fibres lying internal to the longitudinal, nor a layer of longitudinal muscle-fibres underlying the cuticle, but I must confess that my sections are rather thick. There is nothing to remark concerning the parenchyma save that calcareous corpuscles are absent. The cuticle is about 11 microns in thickness on the average.

The excretory system consists on each side of the proglottid of the usual thick-walled dorsal vessel (110 × 44 microns external diameter in immature proglottids, 58 × 44 in mature and still smaller in gravid) and the more internally lying thin-walled ventral vessel, with a lumen three or four times larger than that of the dorsal. The lateral nerves lie just external to the dorsal vessels.

The further development of the mature proglottid into the gravid may be described very briefly. My material being scanty I cannot relate with certainty the probable process of fertilization, but it appears possible that this is effected by the short cirri ejecting spermatozoa to the exterior and these being at once swallowed by the muscular extremity of the vagina, the opening of which lies at the base of the cirrus. It appears improbable that the cirrus can penetrate into the vagina in the usual way, and there is no evidence for intersegmental cross-fertilization and still less for fertilization between different worms. Which ever is the method adopted, the fertilized eggs soon pour into the uterus and its diverticula, which become enormously distended. The median portion of the uterus soon comes into contact with the ventral body-wall (fig. 16, Pl. 12), and this forms a very definite pore or rather slit through which the uterus ruptures and the eggs are expelled in great quantities. The free eggs, preserved in formalin (fig. 13, Pl. 11), measure 45 microns in external diameter (preserved in balsam the same measurement is only 22 microns). They are spherical, the shell being thin and the embryo entirely without hooks. Previous to the expulsion of the eggs the internal organs of the proglottid have degenerated rapidly once fertilization is largely effected, and in proglottids which have expelled all their eggs only occasional traces of the testes and vesicula seminalis, a very ragged row of longitudinal muscle-bundles and stray eggs in collapsed uterus diverticula, are to be seen, apart from the parenchyma and cuticle.

The only feature of striking importance in the structure of the strobila is the situation of the vitellaria external to the longitudinal muscle-band, while the other principal organs are medullary—an arrangement of the organs so far not described in any other Proteocephalid or Monticelliid. The significance of this feature and the systematic position of the genus will be discussed below.

Ephedrocephalus microcephalus Diesing, 1850.
(Figs. 21–30, Pl. 13.)

This tapeworm (fig. 21, Pl. 13) is also found in the Pirarará (Phractocephalus hemiliopterus), but in the rectum. Unlike that of Zygobothrium megacephalum, the internal anatomy of this species has been described in some detail, Mola (1906) having had access to material in the Natural History Museum at Vienna. This is fortunate since I was only able to obtain two specimens of the worm from two of the fishes I examined. My description of this species necessarily will be in part supplementary to that of Mola.

External Features.

The larger of my two worms measured about 72 mm. in length and 4 mm. in maximum breadth (in formalin) and the other was slightly smaller. Both worms were, therefore, very small compared with the giant form described by Diesing, 'longit. corp. 1′ et 2″; latit. med. 4‴', but not much smaller than those subsequently described by Mola, stated by him to be 10–15 cm. in length. Both of my specimens were sexually mature, the uteri being well developed half-way down the strobila length and the masses of contained ripe eggs causing the ventral surfaces of the proglottids to protrude considerably (fig. 25, Pl. 13). In Diesing's and Mola's figures, on the other hand, the proglottids are shown as being relatively flat, the eggs presumably having been discharged through the conspicuous uterine pores. It is noteworthy that the maximum breadths of both the strobila and scolex of Diesing's giant specimen were about the same as those of the larger of my two specimens, viz. about 4–5 mm. in all cases.

The scolex of my larger specimen (figs. 22, 23, Pl. 13) is recognizable as resembling the figures of Diesing and Mola, though the expanded lower portion or collar of the scolex has nothing of the regular curves depicted by Diesing nor of the folded 'cerebral cortex' appearance shown by Mola. In truth this expanded condition of the lower part of the scolex is, judging from the condition of the scolex in my smaller specimen (fig. 24, Pl. 13), solely assumed when the scolex is attached and is indeed an additional means of attachment. We have already seen a similar change of form of the scolex during attachment in Zygobothrium megacephalum. The four suckers are apparently quite typical and I could detect no signs of a central sucker or apical organ, but I must add that my limited material prevented me from studying the scolex in sections.

An unsegmented neck region is absent. In the strobila the creases denoting successive proglottids are not conspicuous owing, as Mola says, to the presence of numerous secondary creases. There is no trace of the lappet-formation so conspicuous in Zygobothrium megacephalum. Uterine pores were not formed in my specimens, though the uteri were full of eggs in the more posterior segments. The cirro-vaginal apertures are very irregularly alternate and situated near the anterior borders of the segments.

Internal Anatomy of the Strobila.

The testes are numerous and rather small (about 73 × 44 microns in horizontal sections of gravid proglottids) and, as in Zygobothrium megacephalum, are scattered over the greater part of the dorsal surface of the proglottid; in this species, however, the testes are, as is well known, situated external to the longitudinal muscle-layer, i.e. in the dorsal cortical parenchyma, and are therefore not medullary (figs. 26, 27, Pl. 13). The cirri and vaginae both open into short genital atria and extend inwards from their apertures through the longitudinal muscle-layer and between the dorsal and ventral excretory canals. The cirrus sac is dorsal to the vagina and, in a gravid segment, measures about 450 × 116 microns (in balsam), and has a thin nucleated wall with apparently an underlying layer of muscle. The contained cirrus is thick-walled and coiled and apparently unarmed. In none of my proglottids is the cirrus extruded, but Mola has supplied a figure of one. The vas deferens, on emerging from the sac, becomes, in gravid segments, very convoluted and dilated to form the vesicula seminalis. Since the testes lie on the outside of the thick longitudinal muscle-layer, it follows that fine branches of the vas deferens must penetrate this layer in order to reach the testes.

The vagina is only slightly convoluted in gravid segments and extends inwards in close contact with the inner side of the ventral longitudinal muscle-layer until it reaches the uterus when it turns dorsally to open into the ovarian isthmus, in the manner depicted by Mola (in fig. 27, Pl. 13, the vagina apparently runs ventral to the uterus, but this is not the case, since in the next section it turns dorsally). There is nothing remarkable about the ovary, which is a large mass lying along the posterior border of the segment and wholly internal to the thick longitudinal muscle-layer (Fuhrmann's diagram on p. 98 of his 1925 paper which shows dorsal projections of the ovary through the longitudinal muscle-layer is incorrect according to my preparations). The uterus develops on the ventral side of the ovary isthmus and takes the form of a single large sac with digitiform subdivisions laterally. My material is not old enough to show the actual formation of uterine pores, but doubtless, as in Zygobothrium megacephalum, the median portion of the uterus, when full of eggs, comes into contact with the ventral median subcuticula and ultimately a perforation is formed. The eggs differ markedly from those of Zygobothrium megacephalum in being elongated in form (fig. 30, Pl. 13), much smaller (44×22 microns in formalin), and in possessing a yellowish yolky material lining the inside of the shell and (presumably) operculate openings at the shell extremities. The embryo is hookless. The vitellaria are numerous, small (44×22 microns) bodies lying, as in Zygobothrium megacephalum, outside the longitudinal muscle-layer, but in this species wholly on the ventral side and not laterally and dorsally as in Zygobothrium megacephalum.

The circular band of longitudinal muscles is composed, as in

Zygobothrium megacephalum, of closely compacted bundles of fibres, the bundles only being distinguishable in gravid proglottids. In immature proglottids the band is remarkably thick (fig. 29, Pl. 13) and the dorsal and ventral halves practically meet, so that the ovary and other rudiments occupy very little space. Only in mature and especially gravid proglottids do the ovary, uterus, and vesicula seminalis develop and cause the halves of the band to separate. The excretory and nervous systems are of the normal type. Calcareous corpuscles are not present in the parenchyma.

Apart from the scolex the morphological features of interest in this worm are the cortical situation of the testes and vitellaria and the wholly ventral situation of the latter. The systematic position of this species will be discussed below.

Peltidocotyle rugosa Diesing, 1850.
(Figs. 31–40, Pl. 14.)

This curious tapeworm was stated by Diesing to have been found in the 'Silurus Pintado', otherwise 'Platystoma tigrinum', and the Pseudoplatystoma tigrinum of Cuvier and Valenciennes. In a fish, the native name of which was given to me as 'Surubim', which was caught in the Solimões at Codajaz, I found seven specimens, mostly immature, of a tapeworm which in most respects strongly resembles the Peltidocotyle rugosa of Diesing, though it differs in the very important character of being devoid of the bilocular suckers figured by Diesing. The question of identity of the host thus becomes of importance as evidence for or against the identification of the worm. Evidence in favour of the view that the host containing the worms described below was Pseudoplatystoma tigrinum is afforded by the fact that my sketch of the fish closely resembles the figure of this species given by Schomburgk (1841) and that Goeldi (1898) gives 'Surubim' as the native name of this species. In other parts of the Amazon I found that this fish was known as the Kaparari. According to Agassiz the species named by Spix 'Sorubim caparari' is Platystoma coruscans, a species probably closely related to Pseudoplatystoma tigrinum and possibly identical, since I found that the 'tigerish' markings on fish called 'Kaparari' and 'Surubim' were very variable, though they were usually quite distinct from those characterizing the 'Sorubim' or 'Sudobim'—the modern Pseudoplatystoma fasciatum (Linn.)—a much more common fish than the Kaparari. I have good reason then for assuming that the host containing the worms to be described below was the same species as contained the Peltidocotyle rugosa of Diesing, and this is one piece of evidence that these worms are Peltidocotyle rugosa. Further evidence is to be found in the general resemblance between the figures of Diesing and those supplied by me (the name rugosa being particularly applicable to my specimens) and in the fact that my specimens to a very large extent fit the generic and specific definitions of Diesing, the only essential difference being that, as I have mentioned already, I cannot in my specimens detect any acetabula on the four (or fewer) spatula-shaped areas borne on the apex of the scolex, and it is quite possible that Diesing mistook the areas for suckers of the usual kind or the bilocular variety. I may add that in all I examined twelve specimens of the Kaparari, only one of which contained Peltidocotyle rugosa.

External Characters.

The largest of my seven examples measured 5·8 mm. in length and 1·4 mm. in maximum breadth. Diesing's largest specimen attained a length of about 38 mm. and a breadth of about 3 mm. The scolex region (there is no distinct neck) in my largest specimen measured about 1·4 mm. long, but the scoleces of the other examples were shorter, both relatively to the strobila and absolutely (figs. 31–3, Pl. 14). The scolex consists of a basal, more dilated, and relatively smooth region, the surface of which is marked with relatively few deep longitudinal creases, and an apical more narrow region, the general surface of which is darker in colour than that of the basal and bears more numerous but less deep longitudinal creases. Just below the summit of this apical region are situated usually four (but sometimes, as in two of my specimens, two or three) spoon-shaped areas which may perhaps be regarded as shallow suckers but which are in reality only oval extensions anteriorly of the smooth base of the scolex. In sections (figs. 34, 35, Pl. 14) the base of the scolex is seen to be relatively solid and with a smooth surface, but the apex is spongy in character (except the spoon-shaped areas), is much creased, and is probably retractile within the base to some extent. The scolex is quite unarmed.

The strobila is flat in transverse section and is covered with a thick cuticle showing both primary (demarcating the segments) and secondary transverse grooves. The proglottids, in my young specimens, do not exceed 20–5 in number and, excepting the terminal proglottid, are all broader than long. Anteriorly the outlines of the proglottids are not evident, owing to the presence of the secondary creases, and it is difficult to see the internal organs in whole-mounted specimens owing to the thick cuticle and subcuticula. The cirro-vaginal apertures are marginal, opening near the anterior limit of the proglottid and apparently alternate fairly regularly. My material is too young for uterine pores to be present.

Internal Anatomy of a Mature Proglottid.

Despite the thick cuticle and subcuticula I have been able to observe the general disposition of the organs in the mature terminal proglottid of a worm mounted as a whole (fig. 36, Pl. 14). The testes are numerous (between 200 and 300), measure between 35 and 40 microns in diameter (in sections), and are scattered over the entire dorsal surface of the proglottid between the lateral strands of vitellaria and the anterior edge of the ovary posteriorly. A few testes may lie above the front portion of the ovary. The cirrus sac is very large (266×66 microns) and extends inwards nearly to the middle of the proglottid. The cirrus, when extruded, is only about 160 microns in length but relatively broad (60 microns) so that it presents a stumpy appearance (fig. 40, Pl. 14). It is unarmed. The vagina opens immediately behind the cirrus, pursues a course parallel with the cirrus sac, and bends back from the middle of the segment and runs to the ovary. In fully mature proglottids the vagina becomes greatly convoluted and dilated in the middle line to form a large receptaculum seminis. The ovary consists of two large masses of follicles, one on each side, united centrally. The ovary is dorsally situated in the proglottid but ventrally to the testes when these two organs overlap. The vitellaria lie, as usual, in two lateral strands, the individual vitellaria measuring about 30 microns in diameter.

The remarkable feature about this species is that, as in the genera Monticellia La Rue and Goezeella Fuhrmann, the testes, vitellaria, and ovary are all situated in the cortical parenchyma and not in the very reduced medulla. In this species the cortical area is not demarcated from the medullary by definite layers of longitudinal and circular muscles but mainly by a difference of texture of the parenchyma, the medullary being denser. Longitudinal muscle-fibres are probably present but are not associated to form bundles or even a definite layer, and in my rather thick sections they are difficult to distinguish. Further, this species also differs from Monticellia coryphicephala (Monticelli) in that the vitellaria form, in transverse sections of the segments, definite half-circles embracing the lateral extremities of the medulla and are not preponderantly ventral as in the former species. In Goezeella siluri Fuhrmann the vitellaria are wholly ventral in position. It remains to be mentioned that in all three species the vagina, vas deferens, and cirrus sac are in the medulla.

Though I cut serial transverse sections of portions of at least three individuals yet I could never distinguish dorsal and ventral excretory canals.

Fig. 36, Pl. 14, represents a young mature proglottid, and the most developed proglottids in my material (cut into sections) only differ in the development of a vesicula seminalis and receptaculum. I am consequently unable to provide any description of the uterus and eggs. In the text-figure, however, I have assumed that, as in Monticellia and Goezeella (the other genera of the Monticelliinae), the uterus is cortical.

The re-definition of this genus will be discussed below; meanwhile, the remarkable character of the scolex and the Monticellia-like arrangement of the principal organs in the cortex may again be remarked upon.

Amphoteromorphus peniculus Diesing, 1850.
(Figs. 41–52, Pl. 15.)

This species, according to Diesing, is found in the intestine of Bagrus goliath, the native 'Dourāda' or Dorād[1] (or Adorād in some localities), the Piratinga rousseauxii of Goeldi (1898) and the modern Brachyplatystoma rousseauxii. This fish is a large (my largest measured 123 cm. in length) and not uncommon Siluroid, and every one of the thirteen specimens which I examined contained Cestodes of some kind, and five (caught between Manaós and Parintins) contained the present species in considerable numbers.

External Characters.

My largest specimen is about 5 cm. long and 1·18 mm. maximum breadth, but other shorter and more contracted specimens may be much broader, as e.g. 2·65 mm. in my broadest specimen which was only 1·5 cm. in length. These measurements correspond very closely with those given by Diesing. The scolex, in the contracted and unattached condition (figs. 42, 43, Pl. 15), is, externally, very similar to the drawing provided by Diesing: that is to say, it may be no broader than the adjoining strobila and consists of a circular longitudinally wrinkled thick wall enclosing a cavity, on the floor of which lie four suckers radially disposed. But more usually, in my material, the scolex wall is expanded (figs. 44, 45, 47, Pl. 15) into an inverted cone-shaped structure with a terminal disc (formed from the lining of the cavity) covered with numerous delicate adhesive processes developed from the subcuticula. A similar change of form of the scolex during adhesion has already been noticed in Zygobothrium megacephalum and Ephedrocephalus microcephalus. Diesing's description and figure of the 'bothrial grooves' and acetabula appear, when confronted with my material, to be more fanciful than accurate. I have found it very difficult to observe clearly the suckers in flattened scoleces, either examined in formalin or mounted in balsam, owing to the presence of the numerous fine adhesive processes just mentioned, but I have succeeded to a small extent in two specimens in which the surface of the disc had been scraped with a scalpel. Vertical and transverse sections of the scolex prove that four bilocular suckers, cruciform in arrangement, exist (fig. 46, Pl. 15), each sucker consisting of a larger central cavity and a smaller distal. Diesing is mistaken in recording an 'os terminale' for this species.

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[1] Not to be confused with the 'Dorado' or 'Golden Salmon', a pink-fleshed Characinid also found in South American fresh-waters.

The strobila is distinctly flattened in transverse section and consists of numerous proglottids, all broader than long, though in some specimens a few of the hind segments approach the square in outline. The posterior borders of the segments do not overlap the segments behind. The ventral uterine pores are situated anteriorly in the segments. The cirrus and vaginal apertures are marginal as usual but are, in this species, unilateral (with possibly an occasional exception), being situated on the right side of the strobila and anteriorly in each segment. In very few of the worms in my collection are the cirri extruded, and the cirrho-vaginal apertures are not distinguished by any papilla or other external feature. Atria appear to be absent.

Internal Anatomy of the Proglottid.

The testes are not numerous, being under forty in number in each segment, and are distributed in the medulla between the ovary posteriorly and the anterior border of the proglottid. Laterally they do not extend beyond the ends of the ovary. The individual testes are about 44 microns in diameter. The cirrus sac is large (280 × 116 microns when the cirrus is retracted), extending across from one-quarter to one-third of the breadth of the segment, and possesses a thin though muscular wall. The cirrus when extended (fig. 48, Pl. 15) is of considerable length (about three-quarters of the breadth of the proglottid, i.e. about 0·580 mm.) and has a slightly distended base containing several coils of the ductus. It is unarmed. The vas deferens is very convoluted and becomes dilated to serve as a vesicula seminalis. The vagina opens, like the cirrus sac, directly to the exterior and immediately in front of the cirrus sac. The portion of the wall next the opening is strongly muscular and the remainder of the tube is very convoluted. In older proglottids the median portion of the vagina becomes dilated and serves as a receptaculum. The ovary is of the usual form—two lateral lobulated masses united by a median isthmus. I have not ascertained the details of the ducts in the region of the isthmus, but it must be mentioned that the uterus arises as a forward-growing tube on the ventral side of the ovary isthmus, and that the full-grown uterus has the taenioid form depicted in fig. 49, Pl. 15. Anteriorly the uterus system opens to the exterior via a wide down-growth—the uterine pore.

The vitellaria, unlike all the other organs so far mentioned, are situated in the cortical parenchyma and are in two groups on each side—dorsal and ventral. The dorsal vitellaria are few in number and are situated above the excretory canals; the ventral vitellaria are numerous at the level of the ovary, extending on each side from near the inner end of the ovary to the outer limit of the cortex, but diminish in number anteriorly so that the ventral group of vitellaria is more or less triangular in form (fig. 48, Pl. 15). The vitellaria are of an oval form in transverse sections, and measure on the average 70 × 18 microns. Intra-uterine eggs, when examined in formalin, are of the shape shown in fig. 52, Pl. 15. The embryos are spherical, measure 14–18 microns in diameter, and are devoid of hooks. Surrounding each embryo is an inner envelope with peripheral refringent (yolk) globules, and externally there is a thin outer envelope, measuring on the average 22–30 microns in breadth and 30–40 microns in length.

As already mentioned, the parenchyma is divided into cortical and medullary regions distinguished not only by a difference of texture but also separated by a definite though thin layer of longitudinal muscle-fibres. The fibres show some indication of bundle-formation in places, but the bundles are quite small and not very definite. Though the medulla is small in area compared with the cortical, yet, as already stated, all the sexual organs, save the vitellaria, are included in it, and in this important respect Amphoteromorphus peniculus resembles Zygobothrium megacephalum.

The excretory system consists of the usual two dorsal and two ventral vessels and, in this species, these are connected respectively, that is to say, dorsal to dorsal and ventral to ventral, across the middle line by transverse canals situated immediately behind the ovary in each segment; further, in each segment the ventral canal of the side bearing the cirrus (but not that of the opposite side) opens to the exterior by a short duct running almost vertically downwards behind the ovary (fig. 51, *vvv*, Pl. 15).

Sciadocephalus megalodiscus Diesing, 1850.
(Figs. 53–9, Pl. 16.)

This small species is, according to Diesing, found in 'Cichla monoculus', which is the Cichla ocellaris of Bloch and Schneider. This fish is known on the Amazon as the 'Tucunaré', and although it is abundant I only examined eight examples, and only in one of them (caught at Manaós) did I find five specimens of Sciadocephalus megalodiscus. My largest specimen of this worm is only 3 mm. long (mounted in balsam) with a maximum breadth of nearly 1 mm. and this I have preserved intact (fig. 53, Pl. 16). Diesing's largest specimen was apparently about 9 mm. long. My other four specimens of this worm were slightly smaller than my largest. One consisted of but little more than a damaged scolex and the other three I either cut into sections (the strobilae and one scolex) or mounted portions (two scoleces) in balsam. I obtained a few good sections of the strobila but not sufficient to enable me to describe the anatomy in detail: hence my account is very incomplete.

External Features.

My largest worm contained about a dozen recognizable proglottids, the terminal being twice the length of the preceding and the last six showing egg-containing uteri. The mature proglottids are the broadest (0·813 mm.), and the most posterior is 0·132 mm. long; the gravid proglottids decrease in breadth towards the end of the strobila though they increase in length, but, excepting the terminal, always remain broader than long.

The scolex in one of my smaller specimens was more expanded and therefore more umbrella-shaped (with broad ribs) than the contracted head of my largest specimen. When the 'umbrella' is fully expanded it is more than double the breadth of the strobila. Moderately expanded scoleces (viewed end-on and in vertical section) are shown in figs. 54 and 56, Pl. 16. In the concavity of the 'umbrella' are four large (0·182 mm. in diameter) spherical suckers, largely obscured from view by overlying folds of the umbrella surface, and one smaller (0·132 mm. in diameter) central or apical sucker, with a distinct opening (the 'os orbiculare' of Diesing). There is no trace of a glandular apical organ. A short thick neck region may be said to be present. In the short strobila the marginal genital apertures open usually a little in front of the midway point of the proglottid and appear to alternate very regularly (judging from two of my specimens and from Diesing's figures). The extruded cirri are not prominent. Uterine pores are not visible in my material, though I have no doubt they develop in older specimens (vide fig. 58, Pl. 16).

Some Features of the Internal Anatomy.

The testes are not numerous (I cannot estimate their approximate number) and are situated at the sides of the proglottid, between the central ovary and the lateral vitellaria. The cirrus sac is very small (about 40×26 microns) and the extruded cirrus is short (44 microns) and thick (19 microns) and is slightly armed (fig. 59, Pl. 16). Both the cirrus sac and vagina open into a shallow atrium. The ovary occupies the centre of the proglottis (in transverse sections) and consists of a large number of follicles (fig. 57, Pl. 16). The uterus also consists of a large number of swollen diverticula (fig. 58, Pl. 16) and doubtless bursts through to the exterior in the mid-ventral line when sufficient eggs have collected. The vitellaria are arranged, as in Zygobothrium megacephalum, in two semicircular (in transverse sections) patches lying immediately external to the lateral excretory canals. It is important to note that a definite band of longitudinal muscle-fibres is entirely absent, though individual fibres may be scattered in the parenchyma. There is thus no question as to organs being medullary or cortical in position. I was unable to detect calcareous corpuscles.

The excretory system consists, so far as I am able to observe it, of one vessel only on each side of the body, somewhat ventral in position but rather thick-walled.

This species is an undoubted Proteocephalid, and in virtue of its unusual form of scolex may, like Corallobothrium solidum, perhaps be regarded as representative of a distinct genus.

Proteocephalus macrophallus Diesing, 1850
(syn. Taenia macrophalla Diesing.)
(Figs. 18–20, Pl. 12.)

As La Rue (1914) remarks, this species, like some others, has so far only been known from the brief definition and drawings of Diesing, both almost wholly concerned with external characters. Diesing stated that this species is found in 'Cichla Monoculus' (the modern Cichla ocellaris), and added (1856) that this fish is called locally the 'Jacunda'. I may remark that the fish called 'Jhărkūndar' which I examined on the Amazon was greenish-grey in colour, of slender elongated form, and devoid of the characteristic ocellus at the base of the caudal fin and therefore quite distinct from the Tucunaré which is the native name for Cichla ocellaris. I found thirteen examples of Proteocephalus macrophallus in a single specimen of Cichla ocellaris caught at Manaós, the largest worm measuring 3·068 mm. in length and 0·94 mm. in maximum breadth (in balsam). In none of these worms are the proglottids gravid, and there is no evidence that the worms examined by Diesing (the largest of which measured about 6 mm. in length) bore gravid proglottids. I may also mention that, in addition to Sciadocephalus megalodiscus and the present species, I found in the Tucunaré two other quite distinct species of tapeworm which superficially might be mistaken for Proteocephalus macrophallus, since they are about the same size, but they are distinguished at once by the fact that in both cases the strobila when not more than 8 mm. in length bears fully gravid proglottids. I shall describe these two forms in a subsequent communication.

Since the anatomy of Proteocephalus macrophallus is typically Proteocephalid and therefore of no particular interest, I shall content myself with a brief diagnosis of the species, necessarily based solely on mature but non-gravid specimens, adding remarks where necessary.

Distinguishable segments in strobila of non-gravid worms less than twenty in number (fig. 18, Pl. 12) and the non-gravid strobila may attain a length of 6 mm. All segments broader than long save the last one (or two) and all flat in transverse section. The region of maximum breadth usually occurs in front of the middle of the strobila length. There is no neck or line of junction separating the scolex region from the strobilia, the scolex only being indicated by the presence of four typical hemispherical suckers (*c.* 0·2 mm. in external diameter). There is no apical organ of any description. The marginal cirro-vaginal apertures irregularly alternate. The testes number 30–40 in each proglottid and measure (in surface view) about 44 microns in diameter. The cirrus sac measures about 146×30 microns and contains a long coiled unarmed cirrus. The cirrus sac and vagina both open into a small but deep atrium. The vagina opens ventrally to the cirrus sac and extends on the ventral side without dilatation to the ovarian isthmus. The ovary is a massive organ lying ventrally against the posterior border of the proglottid and consisting of two lateral lobes united medianly. The vitellaria are small bodies (*c.* 22×14 microns) forming two marginal strands lying, with all other organs, internal to the layer of longitudinal muscle-fibres. The longitudinal muscle-layer consists of isolated fibres (insufficient in number to form bundles) lying in a row in the vicinity of transversely disposed fibres, and these, in conjunction with what looks like a thickening of the parenchyma, form an obvious, if thin, line of demarcation between the cortex and medulla. Small calcareous corpuscles appear to be present in the parenchyma. The cuticle is about 7 microns in thickness. Definite excretory canals are apparently absent. The host and locality have already been mentioned.

I must add that a number of items of description not included in the above are only omitted because I was unable, with my limited and largely immature material, to obtain information concerning them.

A PROVISIONAL RECLASSIFICATION OF THE PROTEOCEPHALIDAE AND THE REDEFINITION OF SOME GENERA FOUNDED BY DIESING.

In view of the fact that I have in my collection of Cestodes from the Amazon several scores of new species which I hope to describe during the next few years, it would seem advisable to lay aside the problem of the classification of forms already known until these new forms have been examined. But there are obvious objections to this course, one being that Diesing's genera certainly require redefinition, and such is difficult until it is known to which family they are to be referred. Moreover, there can be no objection to a temporary scaffolding so long as it is recognized as temporary, and so I propose to suggest without hesitation rearrangements of existing groups according to the information I possess at the time, having due regard, however, to the final taxonomic edifice.

The Order Tetraphyllidea, according to recent authorities (Perrenoud, 1931), comprises seven families—Phyllobothriidae, Onchobothriidae, Proteocephalidae, Lecanicephalidae, Cephalobothriidae, Discocephalidae, and Monticelliidae—but for reasons which I advanced in 1927 (Woodland, 1927), and for present purposes, I prefer to modify this scheme by regarding the Phyllobothriidae and Onchobothriidae as one family—the Phyllobothriidae emend—abolishing the family Monticelliidae for the reasons about to be given, and including the Tetrarhynchidae (usually regarded as a distinct order, the Trypanorhyncha), the result being six families, three large and well established and the remainder small and problematical. Now the majority of species belonging to the family Proteocephalidae —the family with which we are now concerned—answer to the definition of the family which La Rue gave in 1911 and 1914, but even at that time there were known to exist three Proteocephalid-like worms which showed two types of organ-arrangement both distinct from the Proteocephalid type, viz. the 'Tetracotylus' coryphicephala first described by Monticelli in 1891, the 'Corallobothrium' lobosum (renamed by Mola Ephedrocephalus lobosus) first described by Riggenbach in 1896 and Ephedrocephalus microcephalus, the anatomy of which was first described by Mola in 1906. La Rue apparently was only acquainted with the first species, which he renamed Monticellia coryphicephala, and, on the basis of its peculiar organ-arrangement, founded a new family for its reception, the Monticellidae. Later, Fuhrmann (1925), who was acquainted with the anatomy of all three worms and in addition with the anatomy of Marsypocephalus tanganyikae (his Loennbergia tanganyikae) which exhibits a third distinct type of organ-arrangement, confirmed this new family, amending the definition to include all the three types of organ-arrangement which differ from that characteristic of most Proteocephalids. This is a brief history of the origin of the family Monticelliidae. To prepare the ground for the arguments to follow, it is necessary briefly to state the nature of these four organ-arrangements known to Fuhrmann (see diagram on p. 199). In the typical Proteocephalid 'Vitellaria, testes, ovary, and uterus [are situated] within the inner longitudinal muscle-sheath' (La Rue, 1914); in Monticellia and Goezeella the testes and ovary are situated in the dorsal cortex, the vitellaria and the uterus in the ventral cortex: that is, all these organs are cortical; in Ephedrocephalus the testes are in the dorsal cortex and the vitellaria in the ventral cortex, but the ovary and uterus are both in the medulla; in Marsypocephalus the testes alone are in the dorsal cortex, all other organs being medullary and as in Proteocephalids. La Rue based his new family on the characters of the genus Monticellia, but Fuhrmann, as already stated, amended the definition to fit the new facts, the principal character in Fuhrmann's definition being that the 'testes occupy a dorsal or dorso-lateral field in the cortical parenchyma only, whereas the female genitalia may be partly in the cortical and partly in the medullary parenchyma' (Fuhrmann, 1925). Now it is fully evident that these so-called Monticelliidae would undoubtedly be regarded as orthodox Proteocephalids but for the cortical situation of the organs mentioned; they show no close affinities with any other

Tetraphyllidean family. It is also evident that the Monticelliid genera are only grouped together on account of the cortical situation of the testes, since the other genital organs exhibit marked differences as regards position, and I cannot think that this one common character, definite and peculiar as it may be, is sufficient to serve as the necessary bond for a new family. And I am confirmed in this opinion by the fifth type of organ-arrangement, now described in fresh-water Proteocephalid-like worms for the first time, seen in Zygobothrium megacephalum and Amphoteromorphus peniculus. In these species all the organs are medullary save the vitellaria, which are cortical, and they are arranged in a concentric fashion rather similar to that seen in most Tetrarhynchidae. According to Fuhrmann's definition these worms cannot be Monticelliids, nor are they Proteocephalids according to La Rue. Are we then to create another new family?

I believe that the best solution of the difficulty is to abolish the family Monticelliidae and to amend the definition of the family Proteocephalidae so as to include all the aberrant forms mentioned. The definition of the family Proteocephalidae will then read as follows: Tetraphyllidea[1] in which the scolex usually bears true suckers,[2] but never proboscides nor phyllidea;[2] the parenchyma is usually subdivided into cortex and medulla by an internal distinct layer of longitudinal muscle-fibres or by a layer of circular fibres or by a difference of parenchymal texture; the ovary, testes, vitellaria, and uterus are usually situated in the medulla but all these organs can in certain genera become cortical; the ovary is always unilaminate; the vagina runs dorsally to the uterus sac; ripe terminal proglottids do not usually separate from the strobila. Usual hosts are fresh-water Fishes, Amphibia, and Reptiles.

Further, regarding 'Monticelliid' species as aberrant forms of Proteocephalidae, it seems best to subdivide the family into

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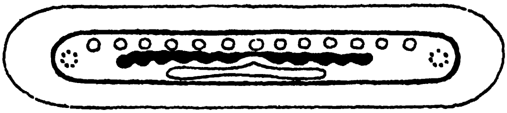
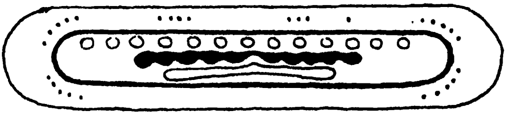
[1] I have attempted to define the Tetraphyllidea in my previous paper (1927).

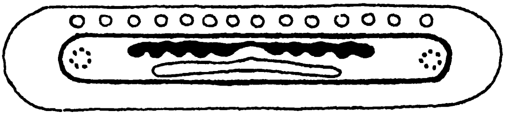
[2] For definitions of these structures and of bothridia, bothria, and suckers I refer the reader to Benham's Chapter on the 'Cestoidea' in Lankester's 'A Treatise on Zoology', p. 115.



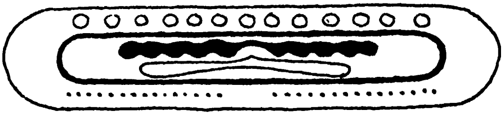
NORMAL PROTEOCEPHALID



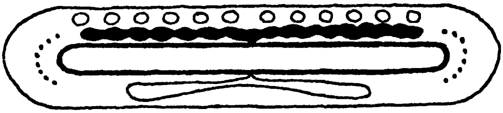
ZYGOBOTHRIUM



MARSYPOCEPHALUS



EPHEDROCEPHALUS



PELTIDOCOTYLE

THE FIVE TYPES OF ORGAN ARRANGEMENT IN THE PROTEOCEPHALIDAE groups according to the types of organ-arrangement shown, and these groups can most conveniently be represented by sub-families. I had reached this conclusion when I learnt of the paper by Mola (1929), who has already proposed the abolition of the family Monticelliidae, the inclusion of 'Monticelliid' genera in the Proteocephalidae, and the formation of sub-families. But his subdivision of the family is rather different from the one I wish to propose, and the sub-families he founded will have to be redefined for my purposes. I propose the subdivision of the family Proteocephalidae into five sub-families, defined as follows:

Sub-family Proteocephalinae Mola, 1929. Proteocephalids in which the testes, ovary, vitellaria, and uterus are not situated external to an internal layer of longitudinal muscles,[1] and the vitellaria are compactly arranged in two marginal strands.

Genera: Proteocephalus, Sciadocephalus, &c.

Sub-family Zygobothriinae Woodland, 1933. Proteocephalids in which the vitellaria alone are cortical in position, and are dispersed and placed dorsally and laterally and sometimes ventrally in the proglottid.

Genera: Zygobothrium, Amphoteromorphus.

Sub-family Marsypocephalinae Woodland, 1933. Proteocephalids in which the testes alone are cortical in position, and the medullary vitellaria are compacted to form two marginal strands.

Genus: Marsypocephalus.

Sub-family Ephedrocephalinae Mola, 1929. Proteocephalids in which both the testes and vitellaria are cortical in position, the vitellaria being dispersed and on the ventral side of the proglottid.

Genus: Ephedrocephalus.

Sub-family Monticelliinae Mola, 1929. Proteocephalids in which the testes, vitellaria, uterus, and the whole or the greater part of the ovary are in the cortex. The vitellaria are dispersed and are lateral or ventrolateral in position.

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[1] This mode of expressing the medullary situation of all the organs is adopted in order to include forms devoid of any distinction between cortex and medulla.

Genera: Monticellia, Goezeella, Peltidocotyle.

It remains to redefine most of these genera.

Proteocephalus Weinland, 1858. Save that it belongs to the Proteocephalinae I do not propose to redefine this genus. Its exact definition cannot be attempted until the validity of other Proteocephaline genera, such as Ophiotaenia and Acanthotaenia, has been determined.

Sciadocephalus Diesing, 1850. Proteocephalinae. Scolex umbrella-shaped with four central suckers and a small apical sucker. Regularly (?)-alternating cirro-vaginal pores. Internal longitudinal muscle-layer absent and no distinction between cortex and medulla.

One species, Sciadocephalus megalodiscus.

Zygobothrium Diesing, 1850. Zygobothriinae. Scolex with four suckers, each with two separate openings. No apical organ. Irregularly-alternating cirro-vaginal pores. Vitellaria situated laterally and dorsally.

One species, Zygobothrium megacephalum.

Amphoteromorphus Diesing, 1850. Zygobothriinae. Scolex with four bilocular suckers, each with a single opening. No apical organ. Cirro-vaginal pores unilateral, on the right side of the strobila. Vitellaria situated dorsally and ventrally.

One species, Amphoteromorphus peniculus.

Marsypocephalus Wedl, 1862. Marsypocephalinae. Scolex with four spherical or ovoid suckers. No apical organ. Cirro-vaginal apertures irregularly alternate. Vitellaria compacted in two marginal strands.

Three species: Marsypocephalus rectangulus, Marsypocephalus heterobranchus, Marsypocephalus tanganyikae.

Ephedrocephalus Diesing, 1850. Ephedrocephalinae. Scolex with four simple suckers. No apical organ. Cirro-vaginal apertures irregularly alternate. Vitellaria ventral and lateral.

Two species: Ephedrocephalus microcephalus, Ephedrocephalus lobosus.

Monticellia La Rue, 1911. Monticelliinae. Scolex with four simple suckers. No apical organ. Cirro-vaginal apertures irregularly alternate. Vitellaria ventrolateral.

One species: Monticellia coryphicephala.

Goezeella Fuhrmann, 1916. Monticelliinae. Scolex with four suckers and a massive collar composed of folds of encircling tissue. No apical organ. Cirro-vaginal apertures irregularly alternate. Vitellaria ventral and lateral.

One species: Goezeella siluri.

Peltidocotyle Diesing, 1850. Monticelliinae. Scolex devoid of typical suckers but possessing two, three, or four spatulate areas which may serve as suckers. No apical organ. Cirro-vaginal apertures more or less regularly alternating. Vitellaria crescentic (in transverse sections) and lateral.

One species: Peltidocotyle rugosa.

ADDENDUM.

In a paper 'On the Classification of the Cestoda' (Southwell, 1929) Dr. Southwell has devoted more than one-third to a lengthy criticism of a number of suggestions which I made in 1929 apropos of the classification of the Tetraphyllidea. I do not propose to reply in detail, but I should like to correct some misstatements in Dr. Southwell's criticism and to explain more clearly one or two of my own arguments. The first misstatement is on his p. 59: 'The characters of the order Tetraphyllidea (sens. nov.) are not given and one is left to guess how this order differs from the other two.' I refer Dr. Southwell to my pp. 539–42 (Woodland, 1927) which, save one-quarter of a page, are entirely devoted to this subject.

With regard to my statement concerning the arrangement of the longitudinal muscles of the Phyllobothriidae, Southwell quotes me as follows: 'the parenchyma is not divided by an internal layer of longitudinal bundles into cortex and medulla, the longitudinal musculature being restricted to an undivided peripheral zone of fibres and bundle of fibres lying immediately internal to the thin circular muscle-layer underlying the cuticle and more or less coextensive with the subcuticula', and Southwell adds that 'It is not clear from this statement whether Woodland considers that the parenchyma is not divided into cortical and medullary portions at all or whether the parenchyma is so divided, but that the longitudinal muscles [sic] are not situated at the junction of the two parts', and he proceeds to show that in certain species I recognize a cortex as distinguished from the medulla. My reply to these remarks is that in some Phyllobothriidae, e.g. in Orygmatobothrium mustoli, Dinobothrium septaria, and many others, there is no line of distinction between medullary and cortical areas of parenchyma, the two areas merging into each other, though it is true that the wide band of scattered longitudinal muscle-fibres and bundles is situated in an outer zone, but this outer zone does not define the 'cortex'; in others, e.g. in Anthobothrium cornucopia, there is also no line of division between cortex and medulla, unless we regard the subcuticula (which contains muscle-fibres) as incorporating the much reduced cortex; in species of Phyllobothrium, on the other hand, an outer zone of parenchyma is definitely separated from an inner zone by a line of circular muscle-fibres, the longitudinal muscle-fibres or bundles being adjacent and extending throughout the cortex. In short, cortical and medullary areas of parenchyma may be recognizable or the reverse, but what is certain is that these two areas in Phyllobothriids are not separated, as they are in Proteocephalids and Tetrarhynchids, by a well-defined row of longitudinal muscle-fibres or bundles. Southwell protests against my assumption that these two areas are demarcated in many Cestodes simply by the band of longitudinal muscles, and states that these two areas are only to be distinguished by the presence of a layer of circular and not longitudinal muscles. But circular muscles are frequently absent, and in such cases I do not think Southwell would hesitate to label the parenchyma lying external to a well-defined layer of longitudinal muscle-bundles as cortex. In other cases, these two areas are only distinguished by a difference of texture of the parenchyma (as in Peltidocotyle rugosa, e.g.) and yet the names 'cortex' and 'medulla' may justly be retained. In short, the terms cortex and medulla only mean in plain language outer zone and inner zone of parenchyma. In many cases they are sharply distinguished by layers of circular and bundles of longitudinal muscle-fibres, and when the circular muscle-layer is clearly visible it certainly affords a more definite line of division than the row of longitudinal muscle-fibres or bundles of fibres, but when circular muscle-fibres are not distinguishable then the row of longitudinal muscles must serve, and, if these are absent, a difference of parenchymal texture, if such exists; in other cases there is no line of distinction between cortex and medulla. As regards my suggestions with respect to the position of the vagina relative to the uterus in the Tetrarhynchidae, on the one hand, and the Proteocephalidae and Phyllobothriidae, on the other hand, and to the quadripartite or x-shaped form of the ovary in the Tetrarhynchidae and Phyllobothriidae and the unilaminate form in Proteocephalidae, I may point out that these distinctions have recently been incorporated in the definitions of these groups by Professor Fuhrmann in his excellent account of the Cestoidea in Kukenthal's 'Handbuch der Zoologie', from which it would appear that they are not as devoid of value as Southwell would have us believe. Finally, I may add that Southwell is again mistaken in asserting that my suggestions for classifying the Tetraphyllidea are solely based on the few species examined by me personally.

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EXPLANATION OF PLATES 11-16.

Reference Letters to Figures.

ant, anterior side; *apo*, apical organ; *at*, atrium; *b*, bilocular sucker; *bd*, spatula-shaped area; *cir*, cirrus; *cort*, cortex; *cut*, cuticle; *cs*, cirrus sac; *duc*, ductus ejaculatorius; *dv*, dorsal vessel; *exv*, excretory vessel; *lm*, longitudinal muscle-layer; *mb*, muscles of sucker; *med*, medulla; *n*, nerve; *ov*, ovary; *post*, posterior side; *s*, sucker; *subcut*, subcuticula; *sv*, sphincter vaginae; *tes*, testes; *up*, uterus pore; *ut*, uterus; *vag*, vagina; *vit*, vitellaria; *vs*, vesicula seminalis; *vv*, ventral vessel; *vvv*, ventral opening of ventral excretory canal.

(All figures drawn under the camera lucida.)

Plate 11.

Figs. 1–13: Zygobothrium megacephalum Diesing, 1850.

Fig. 1.—The entire worm. ×2.

Fig. 2.—Scolex from side aspect, showing two of the bilocular suckers. ×12.

Fig. 3.—Scolex, end-on view of. Openings of suckers distended. This scolex was preserved in an attached condition, whereas that of fig. 2 was unattached. ×12.

Fig. 4.—Scolex, end-on view of. Scolex unattached and contracted. ×12.

Fig. 5.—Scolex in vertical section, showing two of the bilocular suckers in section (the section represented in the diagram of fig. 6). ×18.

Fig. 6.—Diagram of plane of section drawn in Fig. 5.

Fig. 7.—Scolex of young worm attached to mucosa. The base of the scolex is distended and flattened, with a sharp edge. ×12.

Fig. 8.—Portion of mature strobila, showing overlapping posterior edges of the proglottids (the dorsal and ventral aspects are similar). ×12.

Fig. 9.—Sagittal section (in outline) of young strobila to show the overlapping posterior edges of the proglottids. The median band is the medulla. ×18.

Fig. 10.—A similar sagittal section through the elongated terminal proglottids of the strobila. ×18.

Fig. 11.—A young mature terminal proglottid of a young worm viewed from the ventral aspect. ×39.

Fig. 12.—Gravid proglottids showing the taenoid form of the uterus diverticula. ×18.

Fig. 13.—Egg shed from uterus pore of a formalin-preserved worm and sketched while in formalin. ×395.

PLATE 12.

Figs. 14–17: Zygobothrium megacephalum Diesing, 1850. Figs. 18–20: Proteocephalus macrophallus Diesing, 1850.

Fig. 14.—Transverse section through mature proglottid in region of the ovary. ×39.

Fig. 15.—The same in the region of the cirrus sac. ×39.

Fig. 16.—Transverse section through gravid proglottid in the region of the cirrus sac. ×28.

Fig. 17.—The cirrus sac (with cirrus retracted) and vagina opening into the atrium in young mature proglottid. ×88.

Fig. 18.—Young individual of Proteocephalus macrophallus. ×18.

Fig. 19.—Transverse section through entire proglottid in front of ovary. ×88.

Fig. 20.—Transverse section through proglottid in region of ovary. ×88.

PLATE 13.

Figs. 21–30: Ephedrocephalus microcephalus Diesing, 1850.

Fig. 21.—The entire worm. Natural size.

Fig. 22.—The scolex in side view. ×12.

Fig. 23.—End-on view of the scolex when attached. ×12.

Fig. 24.—Side view of scolex when unattached. ×12.

Fig. 25.—Portion of gravid strobila showing uterine swellings (previous to formation of pores). ×12.

Fig. 26.—Transverse section (somewhat oblique) through a mature proglottid in region of future uterine pore. ×28.

Fig. 27.—Transverse section through a gravid proglottid in region of cirrus sac. ×28.

Fig. 28.—Horizontal (oblique) section through gravid proglottids to show form of uterus. ×18.

Fig. 29.—Transverse section through an immature proglottid to show the great thickness of the longitudinal muscle-layer. ×28.

Fig. 30.—Egg (drawn in formalin) in optical section. ×395.

PLATE 14.

Figs. 31–40: Peltidocotyle rugosa Diesing, 1850.

Fig. 31.—The entire worm. ×18.

Fig. 32.—The scolex of another worm in side view showing two spatulate areas. ×28.

Fig. 33.—The scolex of a third worm in side view showing two spatulate areas. ×56.

Fig. 34.—Transverse section across scolex in the thick-walled basal region. ×39.

Fig. 35.—Transverse section across scolex in the upper thin-walled region, showing two spatulate areas. ×39.

Fig. 36.—Terminal segment of a worm. ×39.

Fig. 37.—Transverse section across a mature proglottid in the region of the testes. ×56.

Fig. 38.—Transverse section across a mature proglottid in the region of the ovary. ×56.

Fig. 39.—Portion of transverse section across an immature proglottid to show the cortex and medulla. ×250.

Fig. 40.—The extended cirrus from a proglottid mounted whole. ×250.

PLATE 15.

Figs. 41–52: Amphoteromorphus peniculus Diesing, 1850.

Fig. 41.—The entire worm with contracted (unattached) scolex. ×2.

Figs. 42, 43.—The contracted (unattached) scoleces. ×12.

Figs. 44, 45.—Forms of scoleces when attached to the mucosa. ×12.

Fig. 46.—Outline sketch of the four bilocular suckers on the floor of a scolex. Drawn from a horizontal section. ×28.

Fig. 47.—Sagittal section through the scolex to show the bilocular suckers. ×28.

Fig. 48.—Mature proglottid with partly developed uterus. Dorsal aspect. ×56.

Fig. 49.—Gravid proglottid showing the form of the uterus. ×56.

Fig. 50.—Transverse section through mature proglottid in region of the ovary. ×56.

Fig. 51.—Transverse section of mature proglottid through the uterine pore. ×56.

Fig. 52.—Intra-uterine eggs drawn in formalin. ×395.

PLATE 16.

Figs. 53–9: Sciadocephalus megalodiscus Diesing, 1850.

Fig. 53.—Immature entire worm. ×18.

Fig. 54.—End-on view of the scolex mounted in balsam. ×39.

Fig. 55.—The apical sucker in vertical section. ×56.

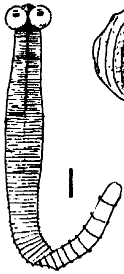
Fig. 56.—Vertical section through two of the four suckers of the scolex. ×56.

Fig. 57.—Transverse section through a mature proglottid in the region of the ovary (anterior portion). ×56.

Fig. 58.—Transverse section through a nearly gravid proglottid. ×88.

Fig. 59.—Extruded cirrus. ×395.

NOTE.—In the text-figure on p. 199 the longitudinal muscle-layer separating the cortex from the medulla shown in the diagram of the type of organ-arrangement found in Peltidocotyle is of course not present in Peltidocotyle rugosa itself, the cortex only being distinguishable by its different texture.



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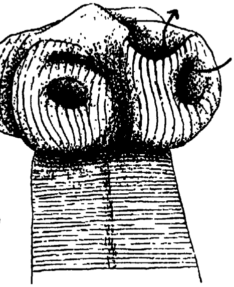

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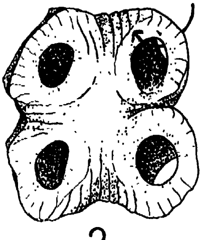

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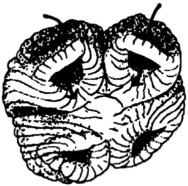

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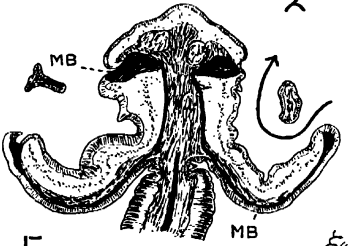

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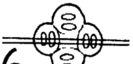

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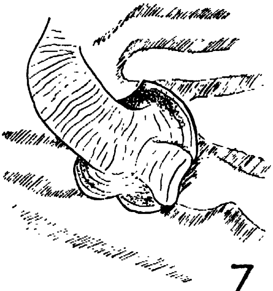

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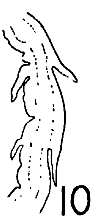

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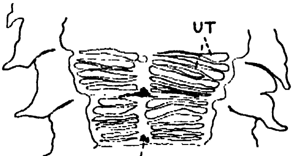

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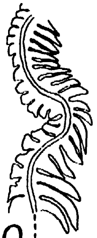

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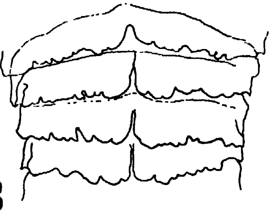

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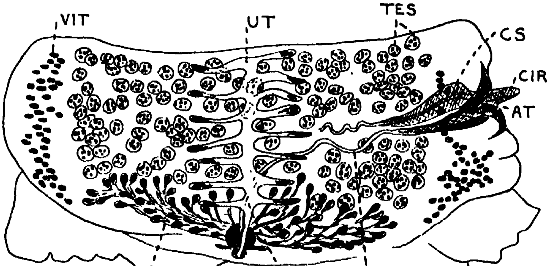

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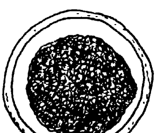

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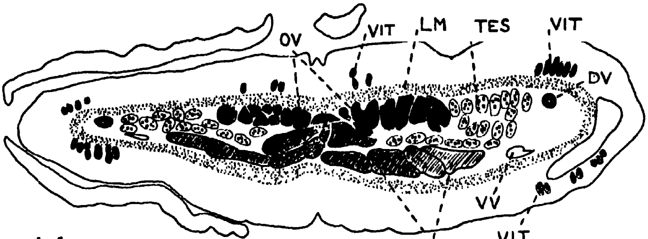

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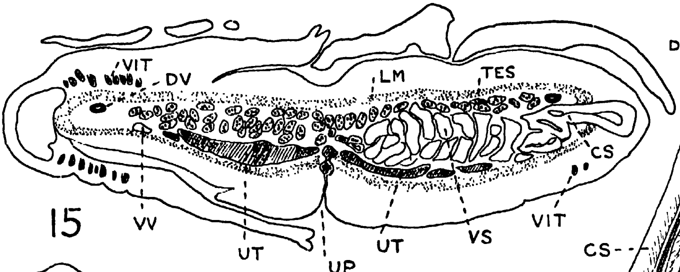

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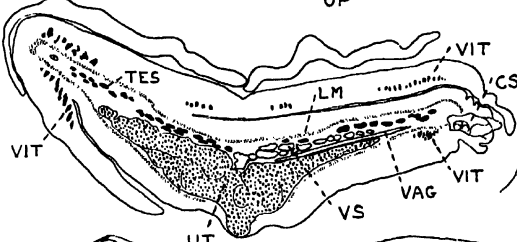

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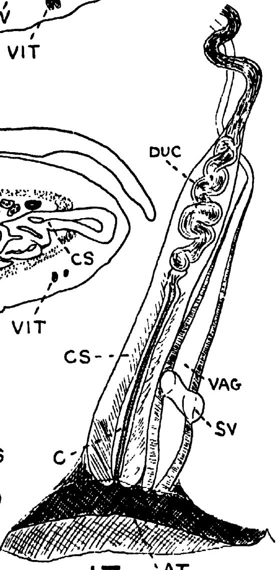

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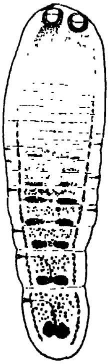

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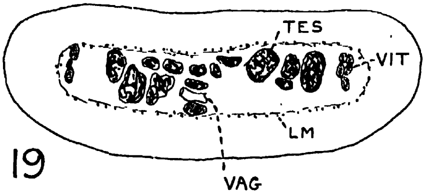

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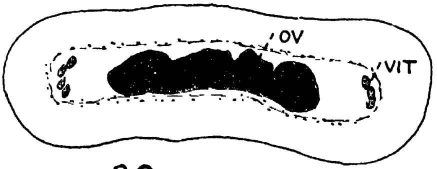

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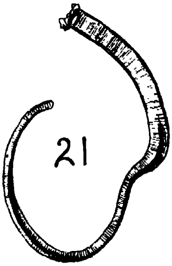

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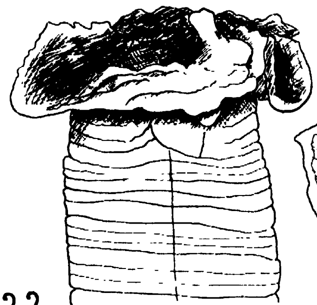

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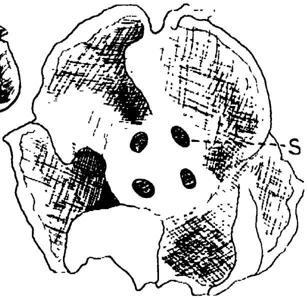

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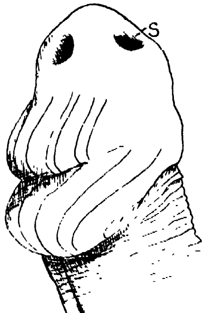

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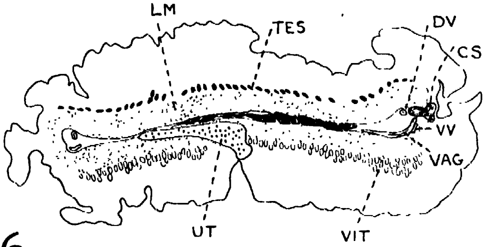

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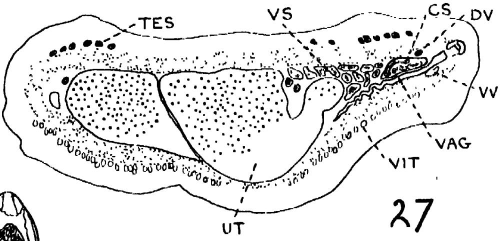

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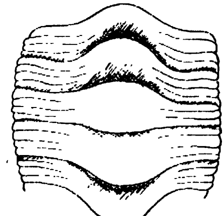

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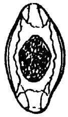

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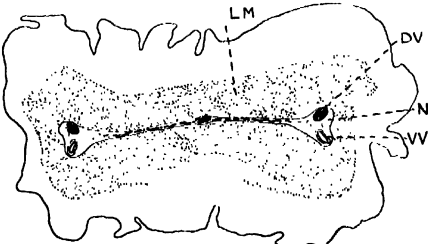

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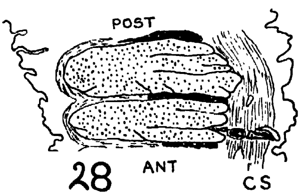

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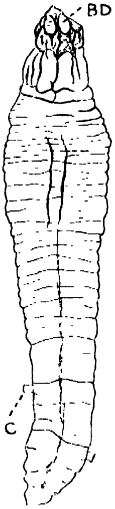

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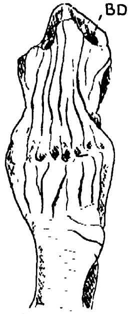

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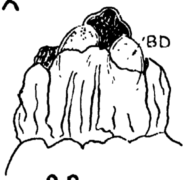

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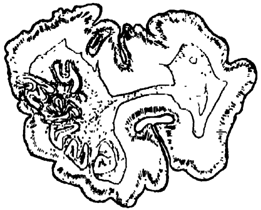

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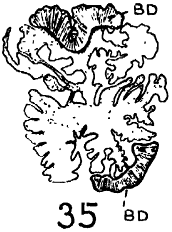

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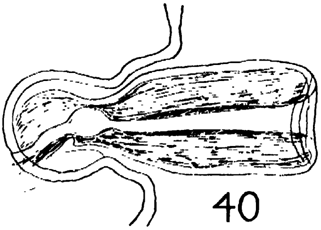

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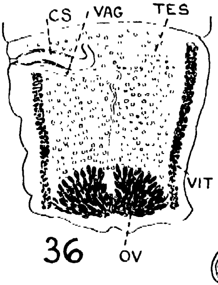

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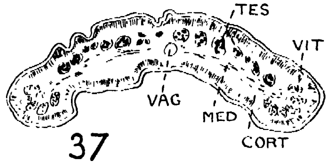

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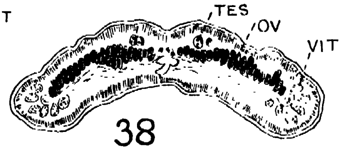

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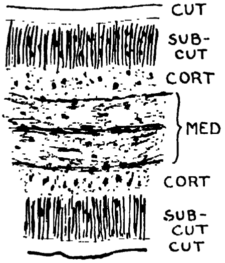

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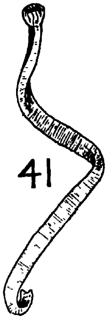

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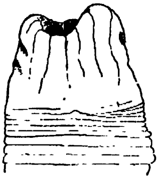

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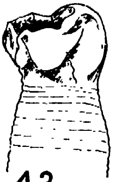

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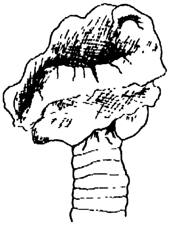

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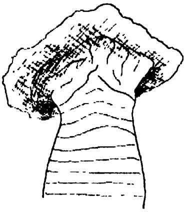

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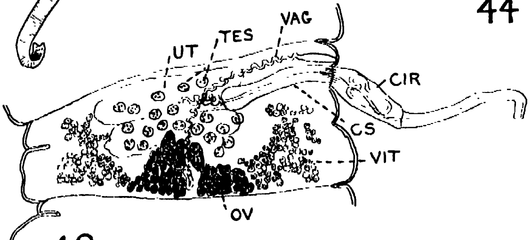

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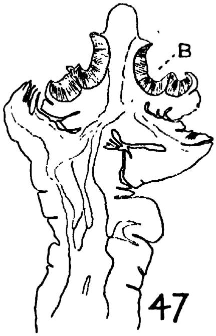

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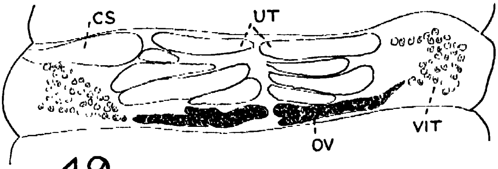

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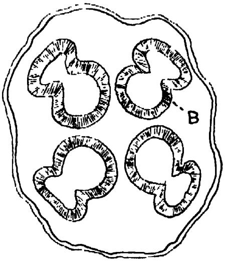

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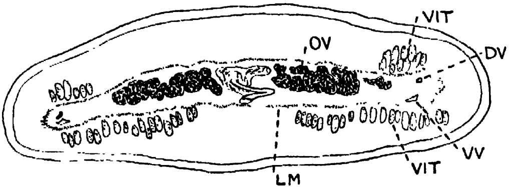

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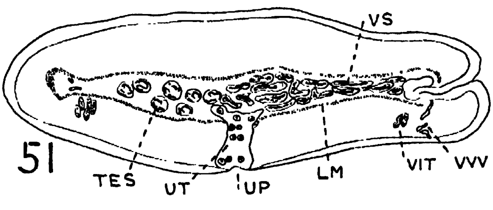

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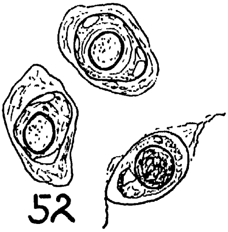

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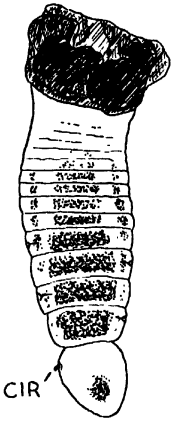

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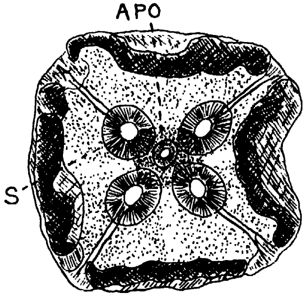

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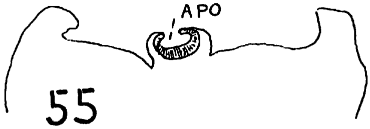

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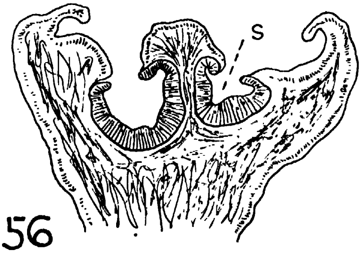

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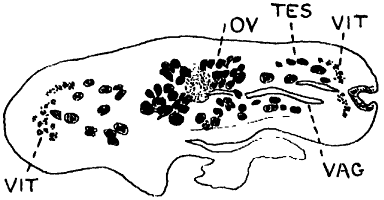

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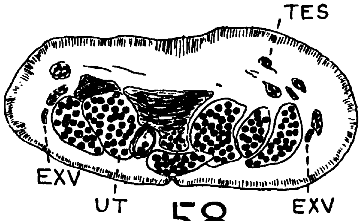

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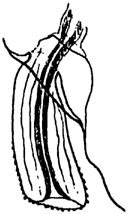

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Notes on the Development of the Chondrocranium of Polypterus Senegalus.

By

J. A. Moy-Thomas, B.A.

Demonstrator in Zoology, the University, Leeds.

With 16 Text-figures.

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INTRODUCTION

THE chondrocranium of Polypterus is far better known in the later stages than in the earlier stages. Pollard (1892) described the cranial anatomy of a half-grown specimen, Budgett (1902) described a 30-mm. larva, and Lehn (1918) gave a detailed account of the neurocranium of a 55- and a 76-mm. specimen. The skull of the adult was first described by Traquair (1871); his description was added to by Bridge (1888), and finally an exhaustive description was given by Allis (1922). The chondrocranium of younger stages is, however, far less well known. The three existing young stages were briefly described by Graham Kerr (1907), and the mandibular and hyoid bars with their associated muscles of the same specimens were described by Edgeworth (1929). De Beer (1926) drew attention to the important morphological characters in the chondrocranium of Polypterus. Thus it can be seen that existing accounts of the development of the chondrocranium of

Polypterus are mainly confined to the older stages, and the development of the young stages is only poorly known.

At the suggestion of my friend and former tutor, Dr. G. R. de Beer, I determined to re-examine Budgett's material and to give a description of the chondrocranium, which would embody all the available information. Working with so few specimens this proved to be rather disappointing, but a few new facts have come to light, which justify a fresh account of these young stages. A really comprehensive review will be impossible until more young larvae of this interesting primitive fish have been collected.

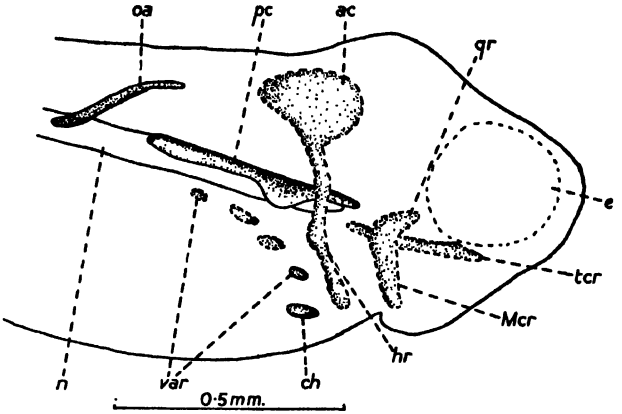
I am greatly indebted to Dr. G. R. de Beer for his criticism and encouragement without which this work would not have been undertaken. This work was carried out partly in the Department of Zoology in the University of Glasgow, where I was privileged to work during a visit. I would like to take this opportunity of thanking Professor Graham Kerr, whose generosity and hospitality alone made this work possible. The reconstructions were made later in the Zoology Department of the University of Leeds, and I desire to express my gratitude to Professor Garstang for giving me every facility during my work and for invaluable criticism. It is, however, to the late Mr. J. S. Budgett, who died from diseases contracted whilst obtaining the material, that I owe my greatest debt.

The material consisted of those three larvae, 6·75, 8·0, and 9·3 mm., briefly described by Graham Kerr (1907), and the 30-mm. larva described by Budgett (1902). Reconstructions were made by the graphical, glass-plate, and blotting-paper-wax methods.

Description of Specimens

Stage 32. 6·75-mm. larva (Text-figs. 1 and 2).

This is the youngest known specimen in which the skeleton is developed. The parachordals, occipital arch cartilages, and the ceratohyals are alone formed of cartilage, the remaining skeleton is merely represented by a procartilaginous aggregation of nuclei. The parachordals are well chondrified and expand into flanges laterally underlying the auditory sacs. They lie

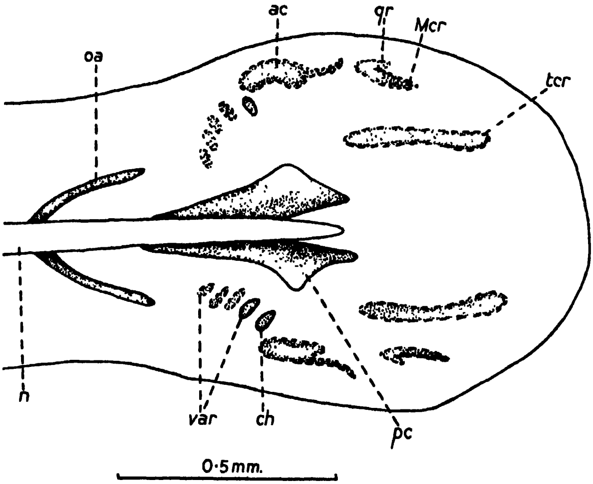


TEXT-FIG. 1.

Polypterus senegalus. Reconstruction of a lateral view of a 6·75-mm. larva. Procartilage with broken outline.

EXPLANATION OF LETTERING OF TEXT-FIGS. 1–16.

abc, anterior basicapsular commissure; *ac*, auditory capsule; *an*, abducens nerve; *aoc*, aortic canal; *ar*, anterior rectus; *bb*, basibranchial; *bcf*, basicapsular fenestra; *bf*, basicranial fenestra; *can*, canal for ophthalmic nerve and superior oblique; *cb 1–4*, ceratobranchials 1–4; *ch*, ceratohyal; *da*, dorsal aorta; *ds*, dorsum sellae; *e*, eye; *eb*, epibranchial portion of ceratobranchial; *ec*, epiphysial cartilage; *ect*, ectethmoid cartilage; *ep*, ethmoid plate; *er*, external rectus muscle; *ff*, foramen for facial nerve; *fpo*, foramen of profundus and oculomotor nerves; *fso*, foramen of superficial ophthalmic nerve; *fta*, foramen for trigeminus and abducens nerve; *fv*, vein foramen; *gf*, glossopharyngeal nerve; *hb 1–4*, hypobranchials 1–4; *hf*, hypophysial fenestra; *hh*, hypohyal; *hn*, hypoglossal nerve; *hr*, rudiment of hyosymplectic; *hs*, hyosymplectic; *h7*, hyoid branch of facial; *ins*, internasal septum; *iob*, inferior oblique; *ir*, inferior rectus; *lao*, lateral aorta; *lbc*, labial cartilage; *lc*, lateral commissure; *llf*, lateral line foramen; *lon*, lamina orbitonasalis; *Mc*, Meckel's cartilage; *Mcr*, procartilaginous rudiment of Meckel's cartilage; *m7*, mandibular branch of facial; *n*, notochord; *o*, occipital bone; *oa*, occipital arch; *oat*, orbital artery; *oc*, oculomotor nerve; *op*, otic process of palatoquadrate; *ops*, opisthotic bone; *pbc*, posterior basicapsular commissure; *pc*, parachordal cartilage; *pf*, palatine branch of facial nerve; *pfc*, prefacial commissure; *pn*, pathetic nerve; *ppp*, post-palatine process; *pq*, palatoquadrate; *pr*, posterior rectus; *prp*, pro-otic process; *pt*, profundus nerve; *pv*, pituitary vein; *pvf*, pituitary vein foramen; *qr*, procartilaginous rudiment of quadrate; *r*, rostrum; *sh*, stylohyal; *sob*, superior oblique; *soc*, supra-orbital closely on either side of the notochord but their anterior ends diverge, leaving the anterior end of the notochord free. There are no separate polar cartilages. The trabeculae are widely separated procartilaginous tracts of nuclei, and lie freely below the brain. The occipital arches are well chondrified; their bases are in contact with the notochord, and they bend forwards and outwards, their anterior ends being unconnected. The auditory capsule and hyoid bar are represented by continuous procartilaginous structures. The mandibular bar is an L-shaped aggregation of nuclei, the dorsal part, according to Edgeworth, representing the quadrate portion, and the ventral, Meckel's cartilage. The ceratohyals are small ventral cartilages lying



TEXT-FIG. 2.

Polypterus senegalus. Reconstruction of dorsal view of 6·75-mm. larva.

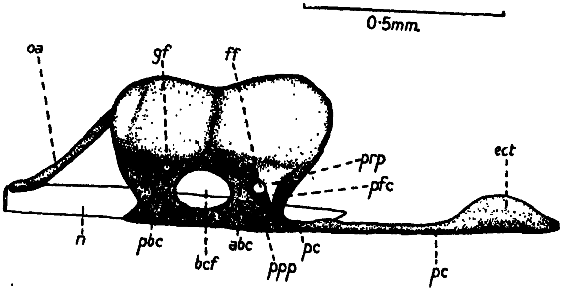
Explanation of Lettering of Text-figs. 1–16 (*cont.*)

cartilage; *sop*, superficial ophthalmic nerve; *sph*, sphenoid bone; *spt*, sphenotic bone; *sr*, superior rectus; *tc*, trabecula cranii; *tcom*, trabecula communis; *tm*, taenia marginalis; *tr*, trigeminus nerve; *ts*, tectum synoticum; *var*, visceral arch rudiments; *vcl*, vena capitis lateralis; *vf*, foramen for vagus nerve; *vn*, vagus nerve.

directly posterior to the lower ends of the hyoid rudiments. Above and behind the ceratohyals lie the rudiments of the more posterior visceral arches consisting of a small chondrification and small tracts of procartilage. At this early stage it is not possible to determine the individual fates of these structures with certainty.

Stage 33. 8·0-mm. larva (Text-figs. 3, 4, 5, 6).

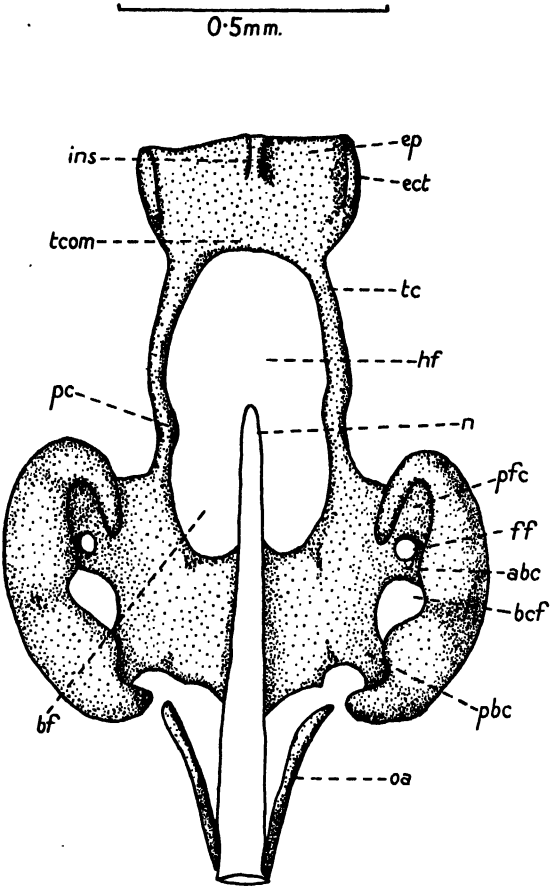
There is unfortunately a very marked gap between the preceding stage and this one. This stage has been damaged on the left side of the head, and the reconstructions of that side have been largely reproduced from the right side. The 8·0-mm. specimen is far more fully developed than the 6·75 mm. The auditory capsule has become fused to the parachordal by the anterior and posterior basicapsular commissures. The foramen for the glossopharyngeal nerve has become separated from the basicapsular fenestra, which is still a fairly wide vacuity. The foramen for the facial nerve is bounded posteriorly by the anterior basicapsular commissure, and anteriorly by the prefacial commissure, which is fully formed at this stage. Laterally to this commissure is a well-marked down-growth, the pro-otic process, which lies outside the vena capitis lateralis. This process, together with the post-palatine process, represented in this stage by a very small lateral knob-like up-growth from the



TEXT-FIG. 3.

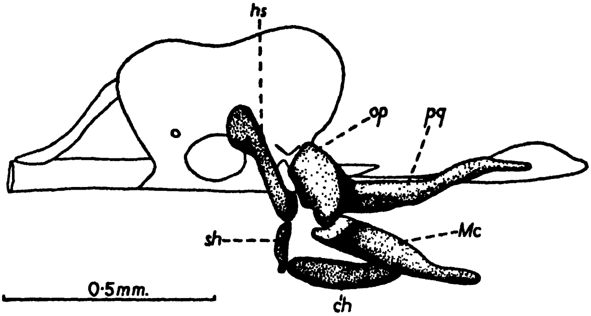
Polypterus senegalus. Reconstruction of lateral view of neurocranium of 8-mm. larva.

parachordal, will form the lateral commissure. The post-palatine process is median and dorsal to the lateral aorta, and



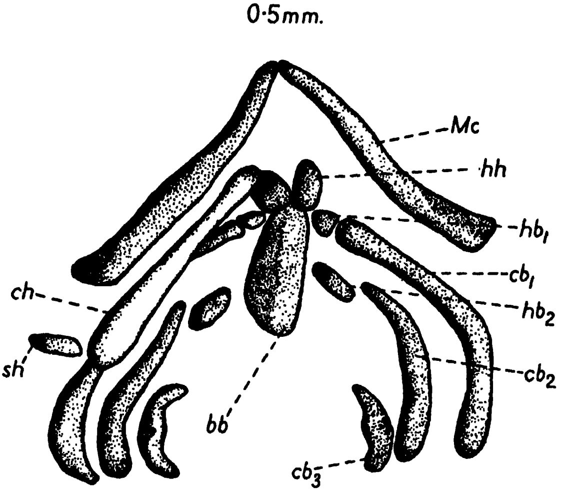
TEXT-FIG. 4.
Polypterus senegalus. Reconstruction of dorsal view of 8-mm. larva.

separates it from the head vein, to which the post-palatine commissure is lateral and ventral. The auditory capsules are roofed dorsally by only a very small quantity of cartilage, and there is no chondrification between the semicircular canals and



TEXT-FIG. 5.

Polypterus senegalus. Reconstruction of lateral view of splanchnocranium of 8-mm. larva.



TEXT-FIG. 6.

Polypterus senegalus. Reconstruction of ventral view of splanchnocranium of 8-mm. larva.

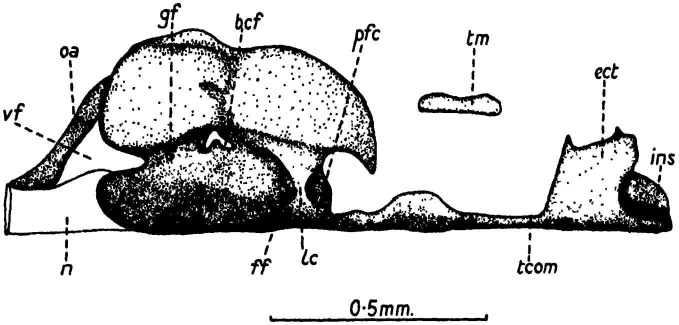
the brain except at the extreme anterior and posterior ends. The notochord still projects freely into the hypophysial fenestra, and the parachordals diverge laterally and join the trabeculae. The connexion of the trabeculae and parachordals is marked by a sinuous curve. The trabeculae are widely separated and fused with one another at the front forming a substantial plate, the ethmoid plate and trabecula communis. The sides of this plate are raised into the lamina orbitonasalis, and centrally there is a small up-growth, the internasal septum. The occipital arches are still only attached to the notochord, their anterior ends lying close but not attached to the auditory capsules.

The palatoquadrate is fully chondrified and has a well-developed otic process (termed metapterygoid process by Sewertzoff, 1926), and a downwardly directed process which articulates with Meckel's cartilage, which is well developed but not attached to its fellow of the opposite side. The posterior end of Meckel's cartilage is expanded laterally, forming a platform. The hyomandibular and symplectic are apparently represented by a single cartilage, and are no longer connected to the auditory capsule. The hyosymplectic is closely associated with the stylohyal which in its turn is associated with the ceratohyal, a well-developed rod of cartilage broad at each end but narrowing in the middle, it articulates with the basihyal through the hypohyal. The first and second hypobranchials are present, and the first, second, and third ceratobranchials; the latter, however, is only slightly developed. The median basibranchial is a broad cartilage stretching only from the posterior end of the hypohyals to the region of the second hypobranchial.

Stage 36. 9·3-mm. larva (Text-figs. 7, 8, 9, 10).

This stage shows considerably more chondrification than in stage 33. The basicapsular fenestra has become reduced to a small slit. The pro-otic process and the post-palatine process have fused to form the lateral commissure which now completely encloses the vena capitis lateralis and the palatine branch of the facial. The prefacial commissure persists. The auditory capsules are more fully roofed, although the two capsules have not yet been joined dorsally by the tectum synoticum. The occipital arches are fused to the back part of the auditory capsules enclosing a foramen for the vagus and hypoglossal nerves. The hind end of the parachordals is not connected to the bases of the occipital arches. The anterior end of the notochord projects freely for some considerable distance and has hardly been surrounded by the parachordals at all. The orbital cartilages or taeniae marginales appear as independently developed cartilages lying approximately equidistant between the auditory capsule and nasal capsule. In the olfactory capsule the lamina orbitonasalis and the internasal septum are a great deal further developed.

One of the characteristics of this specimen is the transient fusion of previously distinct cartilages with one another. The hyosymplectic becomes fused to the palatoquadrate, which is itself, however, free from the trabeculae. The otic process of the palatoquadrate is very well developed. Meckel's cartilages are fused with one another anteriorly, the lateral expansions at their posterior end are more greatly developed. The stylohyal and the ceratohyal are similar to those in the 8-mm. specimen but are both more fully developed, the latter articulating with the hypohyal. All the remaining arches have the hypobranchials and ceratobranchials fused together. The basibranchial is a solid flattened median cartilage with which the hypobranchials articulate.

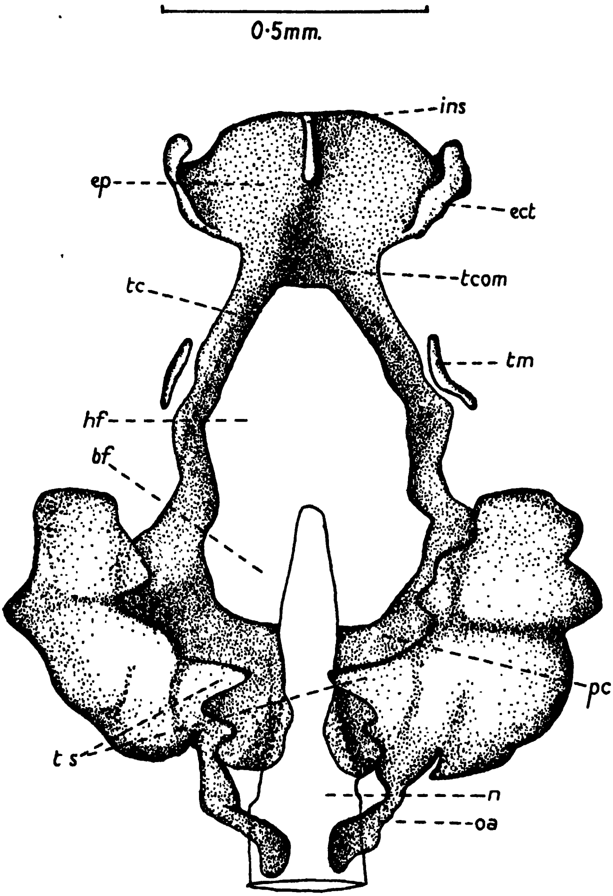


TEXT-FIG. 7.

Polypterus senegalus. Reconstruction of lateral view of neurocranium of 9·3-mm. larva.

80-mm. specimen (Text-figs. 11, 12, 13, 14, 15).

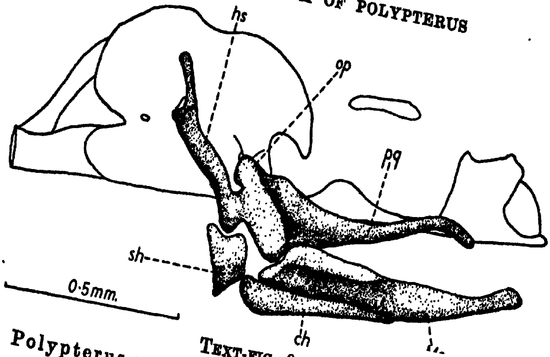
Again there is an unfortunate hiatus between this and the preceding specimen. This specimen is very nearly completely chondrified and differs but little from the 55 mm. and larger



TEXT-FIG. 8.

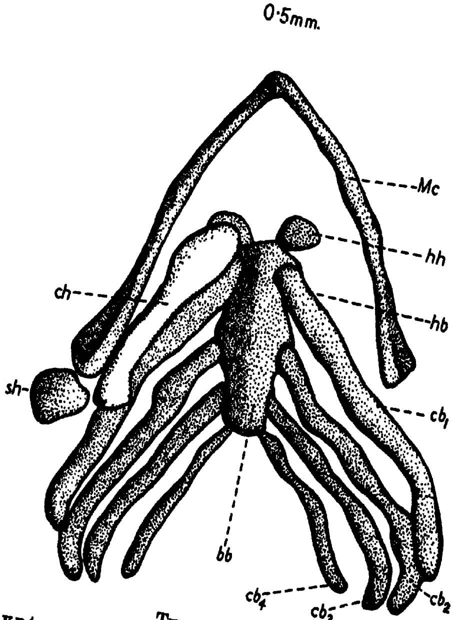
Polypterus senegalus. Reconstruction of dorsal view of 9·3-mm. larva.

specimens of Lehn (1918). Chondrification is, however, not completed in the region of the hypophysis. The hypophysial fenestra is not yet divided by a cartilaginous dorsum sellae



TEXT-FIG. 9.

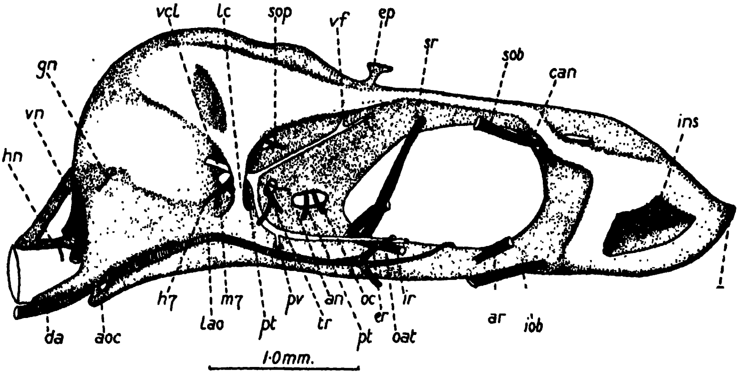
Polypterus senegalus. Reconstruction of lateral view of splanchnocranium of 9·3-mm. larva.



TEXT-FIG. 10.

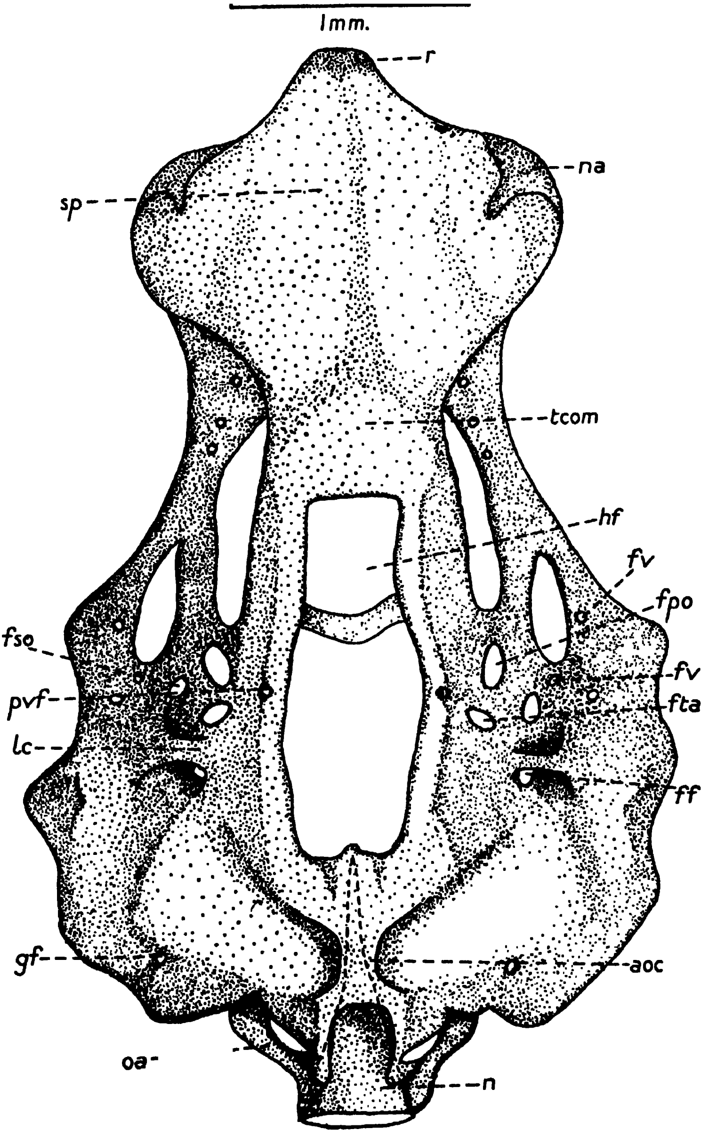
Polypterus senegalus. Reconstruction of ventral view of splanchnocranium of 9·3-mm. larva.

from the basicranial fenestra, as it is in the 55-mm. specimen of Lehn. In the 30-mm. specimen the dorsum sellae is represented by a connective-tissue membrane. Later, according to Lehn, this becomes chondrified, and finally only two very small functionless foramina remain to mark the fenestra basicranialis (Text-fig. 16). The parachordal cartilages now meet below the much-shrunken anterior end of the notochord, and the tip of the notochord no longer projects freely, the connexion between the parachordals being continued in front and below it. The auditory capsules are further chondrified, and the anterior and posterior semicircular canals are enclosed in cartilage much as in a Teleost, and the capsules are connected dorsally with one another by a continuous sheet of cartilage: the tectum synoticum. The lateral walls of the skull have become more chondrified, enclosing the superficial ophthalmic trigeminal, profundus, abducens, and oculomotor nerves. The profundus and oculomotor nerves pass out by the same foramen, and the pituitary vein enters the skull through its own foramen. It was particularly hoped that the early stages might throw some light on this peculiar arrangement by the development of the commissures in this region; but once again it was found that the critical stages do not exist. A true pila pro-otica cannot be



TEXT-FIG. 11.

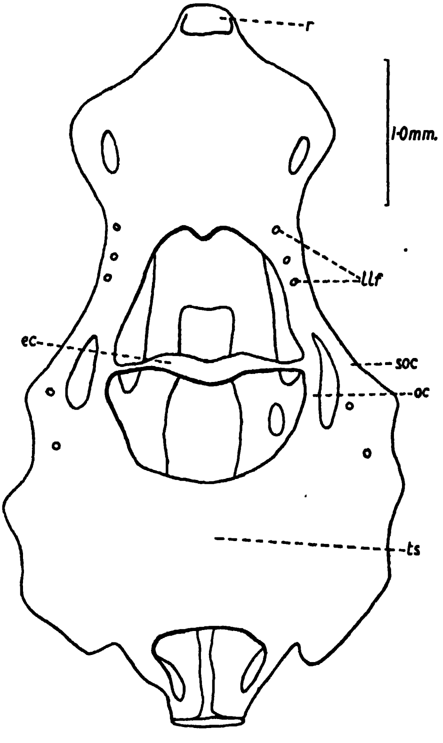
Polypterus senegalus. Reconstruction of lateral view of 30-mm. larva.



TEXT-FIG. 12.

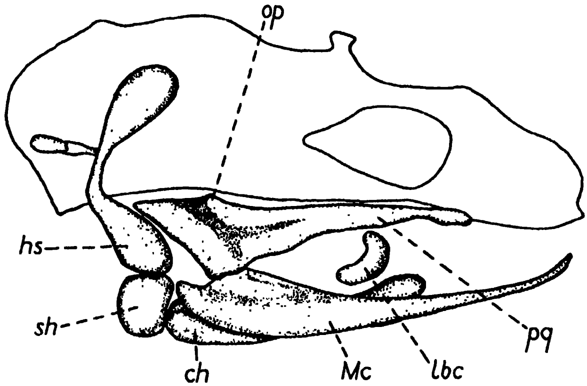
Polypterus senegalus. Reconstruction of ventral view of 30-mm. larva.

present, and the only explanation seems to be the one put forward by de Beer (1926) 'that the pila pro-otica is present separating the pituitary vein foramen from that of the trigeminal nerve, but that in the upper region it has failed to chondrify, thus allowing the profundus to slip forwards and join the oculomotor. The cartilage between the profundus-oculomotor and trigeminal foramen must be secondary.'



TEXT-FIG. 13.
Polypterus senegalus. Reconstruction of dorsal view of 30-mm. larva.

The bases of the occipital cartilages are now joined to the parachordals by a cartilaginous connexion. Down-growths from the parachordals in the occipital region enclose the dorsal aorta in a canal, which ends where the aorta divides. The orbital cartilages have joined both the auditory and nasal capsules and laterally to them two more cartilages, the supra-orbitals, have been formed. The anterior junction of these two cartilages are pierced three times by nerves to the lateral-line system. Dorsally the orbital cartilages are joined by a thin cartilaginous bar, the epiphysial cartilage.

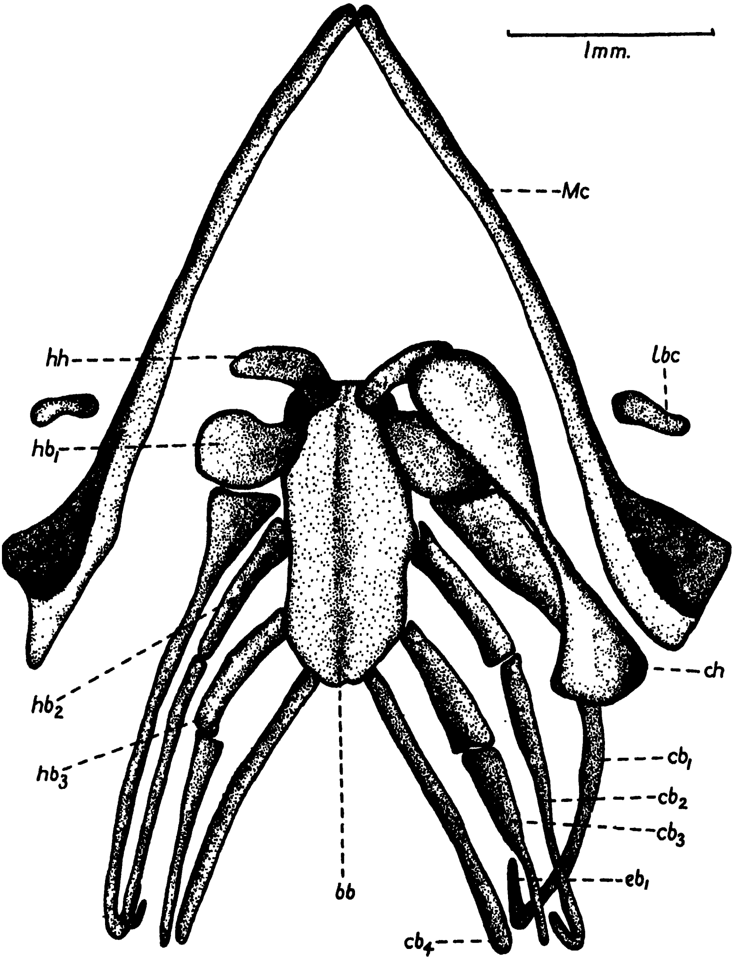


TEXT-FIG. 14.

Polypterus senegalus. Reconstruction of lateral view of 30-mm. larva (simplified from Budgett).

The nasal capsule is a well-chondrified box-like structure completely divided into two anteriorly by the internasal septum. At each posterior dorsal corner there is a canal through which the superficial ophthalmic nerve passes and from which the superior oblique eye-muscle arises. The olfactory capsule has relatively large external apertures and a small anterior rostrum. The trabecula communis has grown backwards, reducing the size of the hypophysial fenestra.

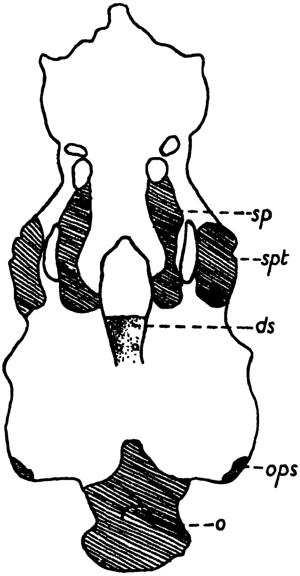
The palatoquadrate has become free from the hyosymplectic and the otic process greatly reduced. The rami of the lower jaw are again separate. Dorsal to Meckel's cartilage and ventral to the palatoquadrate there is a small labial cartilage. The ends of the hyosymplectic have become swollen, giving it the appearance of a bent dumb-bell with a small posterior process for the



TEXT-FIG. 15.

Polypterus senegalus. Reconstruction of ventral view of 30-mm. larva.

external gill cartilage. The ceratohyal has also become greatly expanded at its extremities. The first three hypobranchials and ceratobranchials are no longer fused; but the fourth is still represented by a continuous cartilage. The first hypobranchial and the anterior end of the first ceratobranchial are enormously expanded, and the posterior ends of the first and second ceratobranchials are beginning to bend dorsally forwards, forming an epibranchial portion. The completion of the epibranchials and pharyngobranchials only is necessary before tho adult stage is reached (Allis, 1922), the last bar being permanently unconstricted.



TEXT-FIG. 16.

Polypterus senegalus. Reconstruction of ventral view of 76-mm. larva (simplified from Lehn).

Text-fig. 11 represents this chondrocranium with the main nerves, blood-vessels, and eye-muscles reconstructed. Very little can be added to the excellent accounts of Pollard, Lehn, and Allis. Only the head vein and palatine branch of the facial nerve pass through the jugular canal. The former divides into a dorsal and ventral branch, the latter giving off the pituitary vein. The dorsal aorta divides into the lateral aortae which pass outwards and forwards, give off the orbital artery, and run alongside the trabeculae, and enter the cranial cavity dorsally to them near the optic nerve, as described by Allis (1908).

The posterior, superior, and inferior rectus muscles arise together, but the anterior rectus is attached farther forward to the trabecula communis. The attachment of the inferior oblique is very low down on the trabecula, and the superior oblique arises from the ophthalmic canal.

Discussion of Results.

The phylogenetic position of Polypterus has long been one of the major problems of fish classification. The older view of Huxley (1861) that Polypterus was a Crossopterygian has lost much ground in favour of the theory of Goodrich (1907, 1909, 1928) that Polypterus is an Actinopterygian, possibly a Palaeoniscid. To generalize from such fragmentary evidence as has been put forward in this paper would be impossible, nevertheless comparisons are always a temptation. The following remarks must therefore be taken only to show in what way the chondrocranium of Polypterus resemble those of other fish during its development, but not necessarily as establishing any bond of union between them. Authorities for the developments of Scyllium, Acipenser, Lepidosteus, Amia, and Teleosts are not cited in detail, but a list of the literature consulted is appended at the end.

The chondrocranium of Polypterus is platybasic, and consequently during development it shows a strong resemblance with other platybasic skulls in the general arrangement of the cartilages, particularly in the wide divergence of the parachordals from the notochord. The parachordals of Acipenser, however, do not show this divergence, and connexion between them in front of the notochord is established early. The parachordals are formed before the trabeculae and there are no separately developed polar cartilages, as in Lepidosteus and Elasmobranchs. The trabeculae fuse anteriorly to form the floor of the skull, the trabecula communis, and from the centre of the ethmoid plate a high internasal septum is formed as in Amia and Lepidosteus. In possessing both a prefacial and a lateral commissure, Polypterus is unique among living fishes, the condition is found, however, in the Palaeoniscoids. The formation of the lateral commissure from the pro-otic and post-palatine processes without a basitrabecular process is also unique. The head vein and palatine nerve only pass through the jugular canal, the orbital artery being given off by the lateral aorta considerably farther forward. The pro-otic process forms before the post-palatine process as in Amia, thus differing from Acipenser, Lepidosteus, and Salmo. The peculiar arrangement of the oculomotor and profundus nerves and the absence or partial absence of the pila pro-otica have already been discussed.

The occipital arch remains independent till comparatively late in development, and in so doing bears a very strong resemblance to Amia, whereas in other forms it grows up from the posterior end of the parachordals. The formation of the pro-otic bridge late in development is also a characteristic of Amia and Lepidosteus.

The early appearance of the quadrate portion of the palatoquadrate is only known elsewhere in the Teleosts. The large otic process of the palatoquadrate found in the young larvae, but gradually diminishing in the later stages, has a small homologue in Amia. The hyoid and symplectic remain fused as in Amia and Selachians. The temporary fusion of hyosymplectic and palatoquadrate is reported to occur also in Clupea and Syngnathus. During development, therefore, it is with Amia particularly, and Lepidosteus, among living fishes, that the chondrocranium of Polypterus shows most resemblances. These marked Actinopterygian similarities form additional evidence in support of Goodrich's theory.

It is unfortunate that the study of the development of the chondrocranium does not throw any direct light on the problem of the absence of the myodome in Polypterus. As Watson and others have shown, a myodome was present in the Palaeoniscoids, and some of their descendants (Amia and most Teleosts) have retained it, while others (Acipenseroids, Lepidosteus, and some Teleosts) must have lost it. Polypterus possesses a subpituitary space not unlike that found in Acipenser or Lepidosteus, and therefore, if the latter have lost the myodome, its loss by Polypterus presents no insuperable difficulty. Indeed, what evidence there is goes to show that this is the most reasonable interpretation. The presence of a myodome is associated with the possession of a large and active eye. But the eye of Polypterus is small and embedded in a closely fitting and bony orbit. Indeed, the modest nature of the muscular requirements of the eye in Polypterus, is shown by the fact that the origin of the anterior rectus muscle is shifted forwards, along the side of the skull. The muscle is consequently shorter than it would be if it originated from the same point as the other recti muscles. Yet in fishes with a fully developed myodome, it is this anterior rectus muscle which (together with the posterior rectus) elongates so considerably that the myodomic space is required in order to accommodate it. The reduction in size of this muscle in Polypterus, therefore, suggests that the myodome has disappeared because it is no longer required.

SUMMARY.

The chondrocranium of the existing specimens of the larval Polypterus have been redescribed, and the earlier specimens figured for the first time. The development of the following structures have not been described previously in Polypterus:

1. The lateral commissure which forms first from the prootic and then the post-palatine process.
2. The taenia marginales, which arise as distinct cartilages.
3. The development of the visceral arches and the temporary fusion of the first and second hypobranchials and ceratobranchials.

This development is then briefly compared with those of Scyllium, Amia, Lepidosteus, Acipenser, and Teleosts; a strong likeness being shown between it and that of Amia. The view of Goodrich is thus afforded additional support.

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The Development and Homologies of the Mouth-parts of the Head-Louse.

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With 10 Text-figures.

INTRODUCTION.

IN 1903 Cholodkovsky published his work on the development of the mouth-parts of Pediculus. He asserted that the mandibles and the maxillae appeared in the germ-band at an early stage, but subsequently disappeared, the stylets and the stylet sheath of the adult animal being only a modified labium. This view was criticized by Enderlein (1904) who studied, by dissection, the mouth-parts of several members of the Siphyuculata. According to him the mouth-parts of the adult louse are homologous with the maxillae, hypopharynx, and labium of other insects. He further stated that the mandibles were rudimentary in Pediculus, but persisted in Haematopinus. Thus the views of Cholodkovsky, based on embryological evidence, were diametrically opposed to those of Enderlein, based on comparative anatomy, but no attempt had been made to reinvestigate the development of the mouth-parts of the louse.

The present investigation is an attempt to settle by confirmation or otherwise of Cholodkovsky's work on Pediculus the problem presented by the conflict of views outlined above. In it no attempt is made to deal with a detailed study of the structure of the mouth-parts of the adult, as their structure has been thoroughly investigated already, only their development and homologies are considered. The work was done only on the head-louse (Pediculus humanus, var. capitis).

My thanks are due to Mr. D. R. R. Burt, Lecturer in Zoology, Ceylon University College, for the interest he has taken in my work. I am indebted to my brothers, Dr. S. E. Fernando and Dr. S. F. Fernando for the material which they obtained from the Hospitals in Colombo and Kandy.

TECHNIQUE.

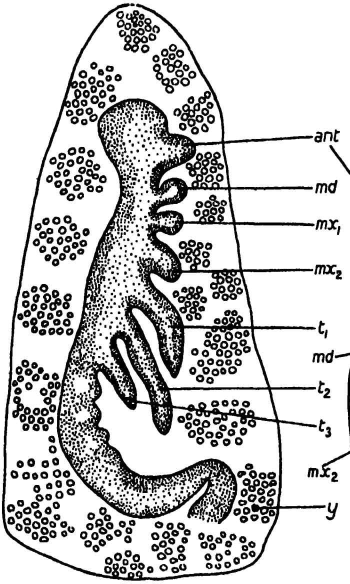
The eggs were fixed in picro-nitric acid and Henning's fluid. The embryos were then dissected out of the egg capsules and were doubly embedded, first in celloidin and then in paraffin, and sections were stained in Delafield's haematoxylin. At the suggestion of Prof. J. W. Munro, of the Imperial College of Science and Technology, London, sections were stained in order to verify the presence of chitinous structures in the mouth-parts. For this purpose Brilmyer's modification of Delafield's haematoxylin and Mallory's connective tissue stain were employed.

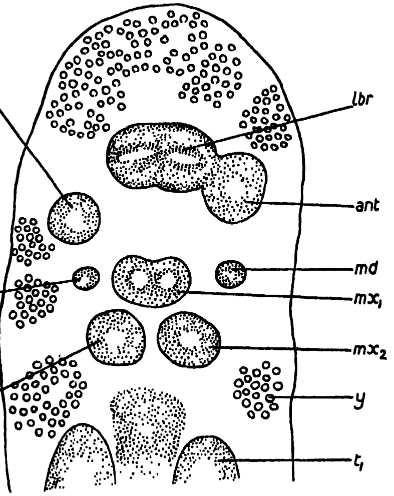
DESCRIPTION.

The limb-buds appear in the germ-band as paired outgrowths The rudiments of the three pairs of thoracic limbs make their appearance first, followed by the labium and the other head appendages. This results in an embryo in which all the appendages of a typical insect are present, though rudimentary (Text-fig. 1). The antennae (*ant*) are the most anterior appendages in the head region; then follow the rudiments of the mandibles (*md*) which are smaller than the antennae; the rudiments of the first maxillae (mx_1) are almost of the same size as those of the mandibles. The labium, representing the second maxillae (mx_2), consists of a pair of rudiments, but these are slightly larger than those of the mandibles and the maxillae. The rudiments of the three pairs of thoracic legs then follow and the abdominal region also shows signs of segmentation, but no appendages are present on the abdomen.

As development proceeds the head region elongates gradually and the antennae become longer. The mandibles and the first maxillae, however, show no signs of elongation, but the labial rudiments grow as rapidly as do the antennae. The differentiation of the thoracic limbs takes place very rapidly, but as we are concerned only with the mouth-parts, we shall confine our attention solely to them.

The stomodaeum arises as an invagination in the region





TEXT-FIG. 1. TEXT-FIG. 2.

FIG. 1. Sagittal section of an early embryo (combined from two sections).
FIG. 2. Frontal section showing union of the first maxillae.

ABBREVIATIONS.

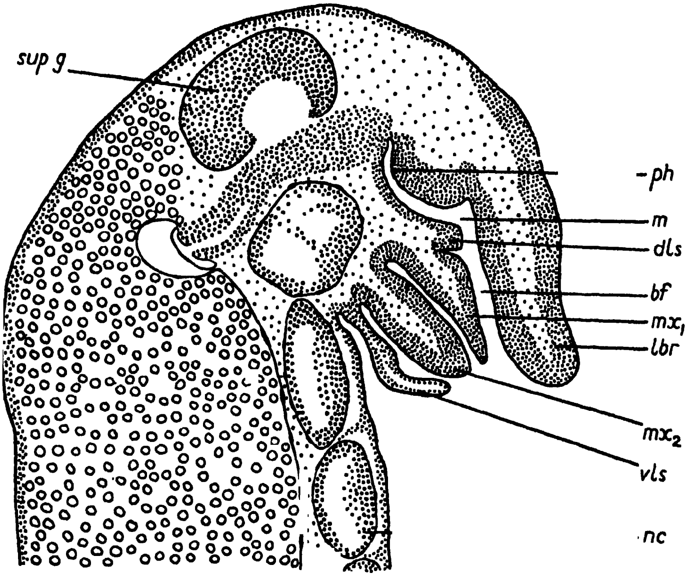
ant., antenna; *bf.*, buccal funnel; *bt.*, buccal teeth; *dls.*, dorsal lip of embryonic stylet sheath; *d.st.*, dorsal stylet; *hyp.*, hypopharynx; *lbr.*, labrum; *m.*, mouth; *md.*, mandible; *mx*$_1$., first maxilla; *mx*$_2$., labium; *nc.*, nerve cord; *oes.*, oesophagus; *Pg.*, Pavlowsky's gland; *ph.*, pumping pharynx; *sal.d.*, salivary duct; *st.s.*, stylet sheath; *sub.g.*, suboesophageal ganglion; *sup.g.*, supra-oesophageal ganglion; t_1, t_2, t_3, thoracic legs; *vls.*, ventral lip of embryonic stylet sheath; *v.st.*, ventral stylet; *y.*, yolk.

between the antennae and the mandibles. The antennae, meanwhile, become displaced so as to assume a more lateral position. Almost on a level with the bases of the antennae, there appear two projections immediately above the mouth. These are the rudiments of the labrum.

With the appearance of the labral rudiments differentiation of the other mouth-parts begins. The mandibles do not appear to grow and remain in their original position (Text-fig. 2, *md*). The first maxillae, however, shift their position more towards the median line, where the two rudiments come so close together that they ultimately fuse into a single projection (Text-fig. 2, mx_1). The double origin of this is always evident in sections from two haemocoelic spaces which it encloses. The fused first maxillae then increase gradually in size. The rudiments of the labium (mx_2) also move towards the median line, but they do not fuse with one another as early as do the first maxillae.

At a later stage the rudiments of the labrum unite with one another, as do the rudiments of the labium. The embryo now presents the following appearance. The labrum is represented by a single structure lying above the mouth, which leads into the stomodaeum. Situated along the middle line posterior to the mouth are the fused first maxillae and the fused labium, each represented by a single projection. During development the former is transformed into the dorsal stylet of the adult, and the latter into the ventral stylet. This transformation takes place during the later embryonic stages. The mandibles, however, retain their original lateral position.

The rudiments of the fused maxillae and of the labium elongate, and at the same time they become withdrawn or sunk into a depression formed immediately posterior to the mouth (Text-fig. 3). The lips of this depression, which surround the first maxillae and the labium, become raised as these rudiments elongate, growth being greater in the ventral lip. The cavity of the depression, now tubular, in which the maxillary and labial rudiments lie, becomes the stylet sac, while the inner surface of the raised lip becomes the stylet sheath of the imago. The result of this development is that the labium appears longer than the first maxillae and the ventral lip of the stylet sheath appears in sagittal section as an appendage formed by the splitting up of the labium. In other words, the ventral lip of the stylet sheath (*vls*) appears like a distinct 'lower lip'. Meanwhile, the labrum has elongated considerably, forming a flap over the other mouth-parts. The original stomodaeal invagination now lies at the base of a canal-like depression which is bounded by the elongated labrum in front and the raised lip and apices of the maxillae and the labium behind. This canal represents the 'buccal funnel' of the adult.



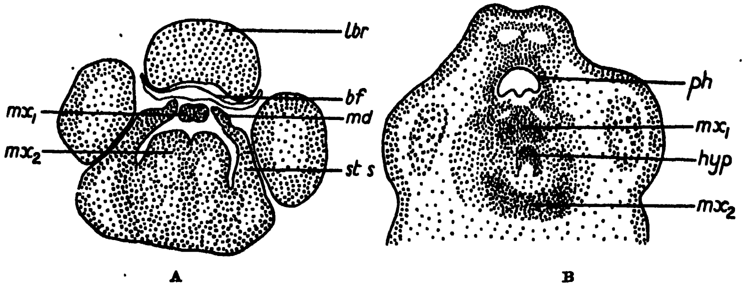
TEXT-FIG. 3.

Sagittal section showing the formation of the stylet sheath.

The portion of the alimentary canal behind the original mouth develops a thick muscular wall becoming the 'pumping pharynx' (*ph*) of the adult.

We have shown that the stylet sheath is formed from the integument surrounding the first maxillae and labium. The mandibles lie on the dorsal lip of the stylet sheath and appear as two small projections directed inwards (Text-fig. 4 A, *md*). Thus the mandibles lie in close proximity to the original mouth opening, but on the smaller anterior (dorsal) lip of the stylet sheath guarding the opening from the buccal funnel into the stylet sac.

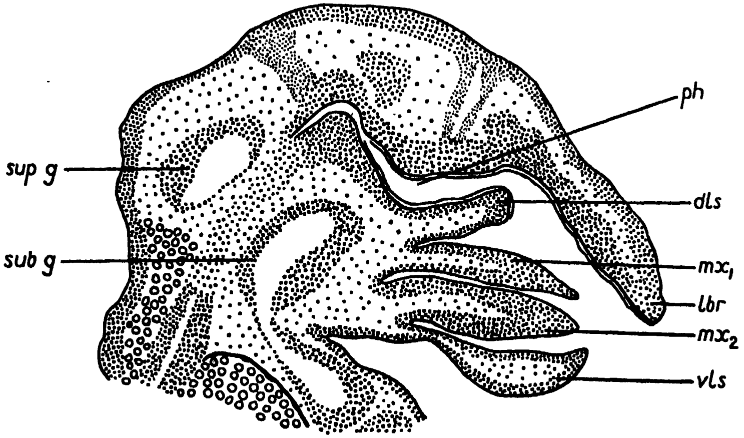
In the posterior region of the head, on a level with the bases of the first maxillae and the labium, there is a small projection lying below the first maxillae (Text-fig. 4 B, *hyp*). It corresponds



TEXT-FIG. 4.

Transverse sections of the head of an embryo.

A. Section passing through the lip of the stylet sheath.
B. Section passing through the base of the stylet sheath showing the developing hypopharynx.



TEXT-FIG. 5.

Sagittal section showing further development of the stylet sheath.

in position with the hypopharynx. Groups of cells being differentiated into the salivary glands lie deeper in the head in the region of the hypopharynx.

The stylet sheath gradually becomes tubular, while the invagination deepens dorsally. Thus the bases of the first maxillae and the labium are gradually carried backwards, and these structures, which at first pointed backwards, now lie horizontally. The ventral lip of the stylet sheath becomes much thicker (Text-fig. 5) and grows out beyond the developing stylets later becoming the ventral and lateral walls of the buccal funnel.

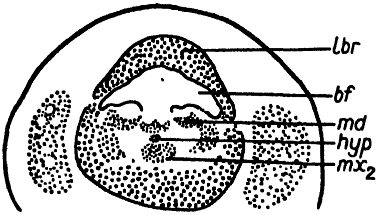
We shall now consider the fate of the various embryonic head appendages. The hypopharynx was shown above to have arisen as a slight projection at the base of the first maxillae. This projection now elongates and assumes the nature of a rod-like structure lying between the first maxillae and labium (Text-fig. 6, *hyp*). The hypopharynx gradually elongates and lies closely applied to the ventral surface of the fused first maxillae. It becomes tubular (Text-fig. 7, *sal.d*) and later on it loses its cellular structure by becoming chitinized as in the imago. We have thus the salivary duct of the adult formed from a modified hypopharynx. At its base lie the salivary glands.

Pavlowsky's glands develop on the sides of the stylet sheath, into which they open at a later stage.

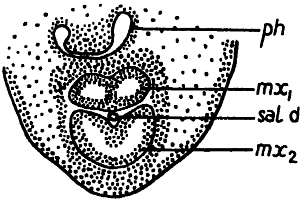
The first maxillae and the labium, as we have seen, were originally paired, their double origin being evident at early stages by the appearance of double haemocoelic spaces (Text-fig. 7). Union of the two rudiments in each case results in the production of a rod-like structure, the first maxillae lying in front of the labium. With the development of the stylet sheath these elongate and become pointed at the tips. An examination of serial transverse sections of old embryos shows a difference in structure in the maxillae and labium. Distally each is single with a single haemocoelic space. Proximally the maxillae remain the same, but the labium divides into an anterior and posterior element (Text-fig. 8 B, *v.st*), and basally the labium is represented by four elements (Text-fig. 8 C, *v.st*).

The mouth-parts of the embryo have now almost assumed the characters of those of the adult. The pumping pharynx and the buccal funnel are lined by chitin. This chitinization also spreads to the maxillae and the labium and, as stated above, to the salivary duct. With the growth of the embryo the epidermal (hypodermal) portions of the maxillae and labium gradually become reduced and withdrawn from the anterior region so that the epidermal elements of the maxillae and labium are only found towards the base of the stylet sheath, while the chitinous covering becomes the definitive stylets of the adult.

The first maxillae become chitinized on the dorsal surface,



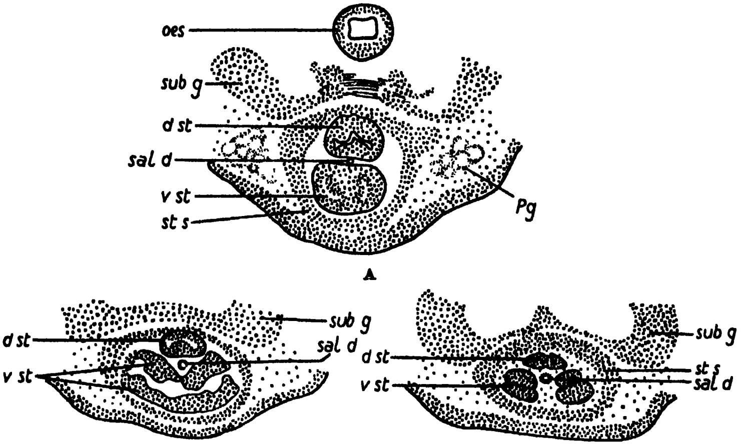
Text-fig. 6.



Text-fig. 7.

Fig. 6. Transverse section of the head of an embryo in the region where the stylet sac communicates with the buccal funnel.

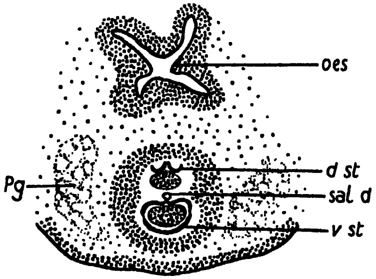
Fig. 7. Transverse section showing the salivary duct.



B Text-fig. 8. C

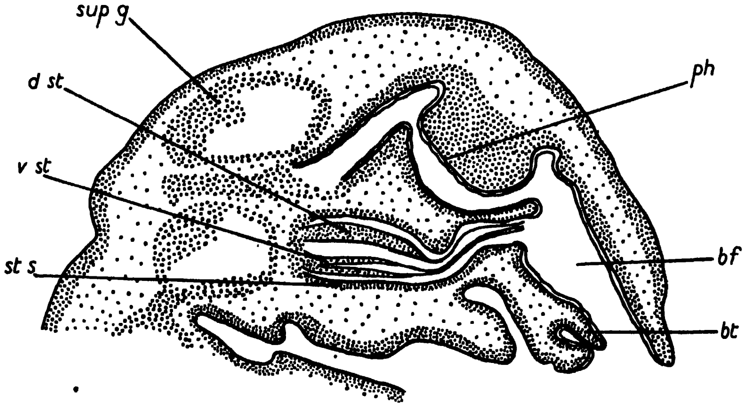
Transverse sections at different levels of the head of a late embryo.

the chitinized areas taking the form of two grooves which diverge at the base of the sheath. In this they reflect the origin of the dorsal stylet from two maxillae. Below the dorsal stylet and almost continuous along its lower surface is the long, slender, chitinous salivary duct (Text-fig. 9, *sal.d*), which is the transformed hypopharynx. Below this lies the ventral stylet (Text-fig. 9, *v.st*) on which chitin is laid down distally on the dorsal and ventral surfaces and proximally the four elements described above are chitinized.



TEXT-FIG. 9.

Transverse section of the head of an embryo before hatching.



TEXT-FIG. 10.

Sagittal section of the head of an embryo before hatching.

The stylet sac lies below the foregut, its base lying close to the suboesophageal ganglion. It opens in front of the pumping pharynx, so that the stylets can be thrust out through the buccal funnel. The buccal funnel was shown to be a canal enclosed dorsally by the elongated labrum and ventrally and laterally by the ventral lip of the stylet sheath. The buccal funnel is lined by chitin. The distal portion of the buccal funnel becomes the rostrum, and we find on it small outgrowths which become the buccal teeth (Text-fig. 10, *bt*).

As regards the mandibles, we were not able to find any traces of them in very old embryos. At no stage were they chitinized, and they disappear completely from the anterior end of the stylet sac.

DISCUSSION.

The present work shows that Cholodkovsky's interpretations of the homologies in the mouth-parts of Pediculus are incorrect, and it is in general agreement with the views of Enderlein. A comparison of Cholodkovsky's and Enderlein's views with that arrived at in this paper shows that the stylet sheath, which Cholodkovsky identified as a portion of the labium, is formed by the integument surrounding the stylets. Enderlein also stated that the ventral portion of the sheath was formed from a part of the labium, but made no reference to the dorsal portion of the sheath.

The labrum was stated by Cholodkovsky to disappear altogether, while Enderlein said that it was represented by a chitinous plate at the tip of the mouth opening. The present work shows that the labrum elongates and forms the dorsal portion of the buccal funnel.

The mandibles, which disappeared according to Cholodkovsky and which were rudimentary in Pediculus according to Enderlein, are here shown to disappear entirely before the insect emerges from the egg. At no stage were the mandibles chitinized.

According to Cholodkovsky the maxillae also disappeared at an early stage, but Enderlein stated that they formed the dorsal stylet. This work confirms Enderlein's conclusion that the maxillae of the embryo become the dorsal stylet of the imago. With regard to the labium, which formed both the stylets and the stylet sheath according to Cholodkovsky, the present paper shows that they are represented by the ventral stylet, as was also asserted by Enderlein.

Cholodkovsky made no mention of the hypopharynx, but Enderlein stated that it was situated between the maxillae and the labium, and we have shown the identity of it with the salivary duct.

SUMMARY.

1. Mandibles, first maxillae, and labium appear in the germ-band at an early stage.
2. The rudiments of the first maxillae unite to form the dorsal stylet, while those of the labium unite to form the ventral stylet.
3. The hypopharynx arises below the first maxillae or dorsal stylet; it becomes tubular and is transformed into the salivary duct.
4. The stylet sheath arises from the integument as a raised lip; it surrounds the dorsal and ventral stylets.
5. The labrum persists as the upper half of the buccal funnel.
6. The mandibles are throughout in a rudimentary condition; they disappear in the adult without becoming chitinized.

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Cytoplasmic Inclusions in the Eggs of certain Indian Snakes.

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With Plate 17.

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INTRODUCTION AND PREVIOUS WORK.

THE present paper, dealing with certain aspects of oogenesis in four species of snakes commonly found in North India, is based on the study of fixed preparations. Attempts made to examine living oocytes were not very successful, and difficulties of procuring suitable material made many repetitions of such attempts impossible. The occurrence and behaviour of some well-known cytoplasmic constituents—Golgi bodies, mitochondria, and yolk bodies—has, however, been revealed sufficiently clearly by classical methods to merit publication, and this account is presented in the hope that it may prove useful to future workers on the oogenesis of snakes.

No work dealing solely with the cytology of snake oocytes has come to the notice of the present writer except some references to snakes in Mlle Loyez's account dealing with reptiles, birds, and cephalopods, published in 1905 (**25**). Among works on other groups of reptiles Bhattacharya's on the oogenesis of tortoises and lizards (**1**) is comparatively recent (1925). This has been supplemented by the publication of small papers dealing with slightly different types from the Zoology Department of the University of Allahabad (**3, 4, 11**). Of the older workers on the subject Munson was the first; his paper on the tortoise, Clemys marmorata, appeared in 1904 (**28**). He was followed by Mlle Loyez referred to above. Since then, Miss A. Thing (**41**), writing on the zona pellucida in turtle eggs, and Bulliard on Emys Lutria (**7**), are the only two names that need be mentioned. Both these workers, as was pointed out in a previous paper (**3**), confined their attention only to certain aspects of chelonian oogenesis.

MATERIAL AND TECHNIQUE.

Snakes, mostly adult specimens, were killed mechanically and the ovary was taken out and fixed immediately. All precautions recommended by Gatenby and Cowdry (**14**) and by Bowen (**5**) for avoiding post-mortem changes were taken. The fixatives successfully employed were those of Da Fano, Cajal, Regaud, Regaud Tupa, Ludford's latest osmic method, Champy, Flemming without acetic, Mann-Kopsch, and Bouin. The snake oocyte has a thick covering of follicle cells which makes it difficult for the fixatives to penetrate to the interior. To obtain good results, the period for which the material was allowed to remain in the different fixatives, and also the subsequent operations (osmication, silver-action, chromatization, reduction), had to be increased to three times the period recommended in 'The Microtomists' Vademecum'. The best results were obtained from the methods of Da Fano, F.w.a., and Ludford's latest osmic method. The Da Fano and Cajal sections were toned by the usual method of Gold chloride and Hypo. Sometimes a 0·3 per cent. solution of ammonium sulphocyanide was added in equal quantity to a 5 per cent. solution of sodium sulphate to make up the hypo. The device of keeping the fixatives at zero degree Centigrade for twelve hours or more was tried and found to improve the fixation. Osmic preparations scarcely stood in need of being bleached by oxalic acid and potassium permanganate.

OBSERVATIONS.

(i) Golgi Bodies.

Zamenis mucosus. Lin.

In the earliest oocyte obtained the nucleus is eccentric and the Golgi apparatus lies on one side of it in a juxtanuclear position. The individual elements, in a Ludford preparation, appear either as curved dictyosomes, or as solid spherical bodies having a lightly staining centre and a heavily impregnated rim, similar in structure to the Golgi elements of the egg of the spider as described by Nath (**31**), by Nath and his collaborators in a number of other animals (**32–4**), by Gresson in Tenthredinids (**16**) and Periplaneta (**17**), and by Gardiner in Limulus polyphemus (**15**) (fig. 1, Pl. 17).

In the next stage the Golgi bodies are spread out in the cytoplasm (fig. 2, Pl. 17), and in fig. 4, Pl. 17, this process of dispersal is seen very near completion. Later the Golgi bodies come closer and arrange themselves in a perinuclear position in a similar manner to that previously recorded for the eggs of frogs, tortoises, birds, &c. This is the preparatory process for the clustering together of these bodies, which is such a marked feature of subsequent stages.

Later stages show the Golgi bodies bigger and more irregular, but a close examination reveals the fact that each irregular individual body is but a collection of many smaller granules whose precise shape is not easily made out. Finally, many of these aggregated bodies come closer together, and form little clumps round the nucleus, some even adhering to its membrane; these latter formations being the result of double clustering. This coming together of the Golgi bodies starts all round the nucleus, whence the process is extended to the cortex (fig. 4, Pl. 17).

Some of the Golgi elements in the early stages swell up and become round. The process of swelling begins when the dispersal of the juxtanuclear Golgi apparatus is not quite complete, and lasts for a short time. The individual swollen bodies ordinarily appear homogeneous, but careful focusing reveals them slightly lighter in the centre than in the cortex (fig. 2, Pl. 17).

Gongylophis conicus. Schneid.

Before the follicular cells are formed round the oocytes in this snake, the Golgi bodies are large and irregular and appear jet black in osmic preparations. The elements are not far apart from each other in the cytoplasm though they were not seen to aggregate in any specific area round the nucleus. In an advanced oocyte they are peripheral in position and lie in closely aggregated clusters. A number of these clusters join together again and form secondary groups of Golgi bodies. This process of regrouping occurs more in the cortical than in the central region of the egg (fig. 8, Pl. 17). The individual Golgi elements contributing to these clusters are derived from two sources: (1) the Golgi bodies originally present in the cytoplasm, (2) the Golgi bodies migrating inwards from the theca and the follicle cells through the zona radiata and becoming indistinguishable on arrival in the cortex from the Golgi bodies of the oocyte. This infiltration of the Golgi bodies from the follicle cells inwards is well seen in fig. 8, Pl. 17 (see paper, **24**). The compound Golgi bodies are present alongside the individual Golgi elements, and when the latter begin to be scarce in late stages the former disappear altogether.

Tropidonotus stolatus. Lin.

Golgi bodies in the smallest oocyte available were aggregated in parts of the cytoplasm; later they became dispersed in the usual way and still later disappeared altogether (fig. 9, Pl. 17). A fully formed egg shows the Golgi bodies again in the cortex, often in small groups of a few individual elements (fig. 10, Pl. 17). In view of the absence of these bodies from previous stages, it is possible that their reappearance in the late stages is due to their infiltration from the follicle cells, which, as has been shown, in another paper (**24**), become periodically active and send numbers of the Golgi bodies into the oocyte.

Tropidonotus piscator. Schneid.

Fig. 11, Pl. 17, is a Mann-Kopsch preparation of the earliest oocyte examined. Golgi bodies lie all round the eccentric nucleus. In a more advanced oocyte, but before the formation of a zona radiata, the Golgi bodies were fully dispersed in the cytoplasm. They were more abundant in the peripheral region, being reinforced by the infiltration of Golgi bodies from the follicle cells.

(ii) Mitochondria.

Zamenis mucosus.

The mitochondria occur as minute granules in the archoplasmic area. Unlike those of Indian tortoises, they are never seen in patches, nor do they become dispersed as such. The mitochondria, like the Golgi bodies, swell up and form round bodies (fig. 2, Pl. 17). This condition is shortlived and in later stages they get more and more minute, settling as a cloudy layer in the cortical region (fig. 4, Pl. 17). This is about the nearest approach to the 'zone mitochondriale corticale' of Van Durme described in avian eggs (**42**). This condition again does not last long, and in a fully grown oocyte the mitochondria become very scarce, though not quite non-existent (fig. 6, Pl. 17).

Tropidonotus stolatus.

The mitochondria were not seen in the early stages; in advanced oocytes they were observed in the cortical region, some of them swelling to form larger spherical bodies.

Tropidonotus piscator.

Mitochondria were observed as minute granular bodies both in the early and advanced oocytes; in the latter stage only in the cortical area (fig. 11, Pl. 17).

Gongylophis conicus.

Mitochondria not observed.

In all cases mitochondria were either granular or cloudy and dust-like. The method advocated by Champy and Bulliard for the demonstration of filamentar mitochondria, consisting of fixation in Regaud's fluid followed by post-chromatization for three months and staining by Champy-Kull method or with iron-alum and haematoxylin, was tried, but no positive results were obtained.

The theca, follicle cells, and zona radiata are characterized by the complete absence of mitochondria. This is noteworthy in view of the fact that not only the presence, but the infiltration of mitochondria from the follicle cells to the oocyte, was noticed by the present writer in tortoises (3) and by Bhattacharya and Das recently in certain other vertebrates (unpublished).

(iii) Yolk Formation.

Zamenus mucosus.

Fatty-yolk bodies are found in early oocytes almost at the beginning of the 'intra follicular growth period'. They occupy the entire cytoplasmic area and are often close to the Golgi bodies (figs. 1 and 3, Pl. 17). The yolk bodies, blackened in osmic preparations, are decolorized in pure turpentine and in xylol leaving behind dark rims, which on close examination are found to be made up of little round bodies, which are interpreted by the present writer as Golgi granules, aggregating concentrically round an area of fat (fig. 5, Pl. 17), a case partly analogous to that obtaining in Indian tortoises (3). There is, however, this difference—that, whilst in the latter the rims are continuous, in snakes they are formed by a number of separate round bodies. Soon after the fatty-yolk bodies arrange themselves in the cortical region, where they begin to disintegrate and disappear altogether in later stages. The formation of the albuminous yolk-bodies starts at this stage near the centre and spreads cortexwards, where they ultimately settle down. The individual elements are much smaller than their prototypes in tortoises (fig. 6, Pl. 17).

Tropidonotus stolatus.

Fatty and albuminous yolk were noticed, their first appearance and subsequent behaviour agreeing with the process in Zamenis mucosus (figs. 9 and 10, Pl. 17).

Tropidonotus piscator.

No fatty yolk was observed. Albuminous yolk was noticed in advanced oocytes; often in vesicles, the latter growing smaller as the yolk granules grew bigger.

Gongylophis conicus.

No yolk of any kind was observed.

DISCUSSION.

The behaviour of the Golgi bodies in the four species of snakes examined was not very dissimilar. In some cases their tendency to swell in early stages has been mentioned. This was most marked in Zamenis mucosus. This tendency coincided in part with the appearance of fatty-yolk bodies in the oocyte. The two bodies, however, remained distinct chemically: the swollen Golgi bodies remaining intact after long treatment with turpentine, whereas the central material of the fatty-yolk bodies dissolved in the liquid after comparatively shorter periods. The following observations of Nath (**30**) further show that Golgi bodies need not necessarily change their chemical nature in the process of swelling:

'In several other cases, however, e.g. Ascidians (Hirschler 1915) Julus (Nath in press), the Golgi elements simply swell up without in any way changing their chemical nature, and it is very likely that in such forms the swollen Golgi elements will shrink to their normal size during embryogeny and will not be used as yolk. The possibility, however, remains that when such swollen Golgi elements shrink some of their material will pass into the cytoplasm and may be used up in the metabolism of the cell.'

It is not possible to suggest that these swollen bodies contribute to the origin of the fatty yolk; but considering their complete absence in later stages, when the fatty yolk is still developing, it may be that they contribute in some way to its growth.

The clusters of Golgi bodies, already alluded to, were found in all the types and with most of the fixatives. It is improbable that they would merely be the result of individual elements running together under the influence of fixatives, as may be suggested. Somewhat similar phenomena occur in Limulus polyphemus, where Gardiner (**15**) mentions the fact of the yolk nucleus fragmenting and the dictyosomes coming to lie at the rim of the oocyte in 'little groups or islands'.

Individual Golgi bodies have been noticed to have a fairly uniform appearance—an argentophilic or osmiophilic rim surrounding a comparatively clear vesicular area. According to Parat's 'Vacuome Theory' these bodies, in part at least, are artifacts, the clear central area in each of these bodies being alone believed to represent the true (vacuolar) Golgi element. This view is no longer tenable. The vacuoles and the Golgi bodies have been observed as separate structures in the male germ-cells of a number of animals, including man, by Gatenby (**12, 13**), Hirschler (**22**), Monne (**27**), and Voinov (**43**); and in the oocytes of Lumbricus by O'Brien and Gatenby (**37**), of the pigeon by Bhattacharya and Das (**2**) and of Rana tigrina, Ophiocephalus punctatus, and Rita rita by Nath and Nangia (**36**); while Harvey from a study of Lumbricus (**19**) concludes that 'the vacuome in this material is the cell's reaction to neutral red and is not present in the unstained egg' and that 'it arises in close relation to the Golgi apparatus, but in later phases of staining wanders away from it'. The last-mentioned author, in a later paper on Echinoderm oogenesis (**20**), thinks, however, that the vacuome may be originally present in the oocyte as something similar to it in size and position appears after osmic acid staining in young oocytes. In the absence of any examination of living snake oocytes it is not possible to say if the neutral red staining vacuole exists independently in the cytoplasm or in any relation to the Golgi apparatus or does not exist at all, but the identity of the latter should not be in doubt. In view of recent work Golgi bodies having a chromophobic centre and chromophilic rim can only be regarded as merely dual-structured, and their interpretation as an association of a vacuome with an osmophilic and argentophilic cortex cannot be justified.

In some stages of Tropidonotus stolatus swollen mitochondria and albuminous yolk remain side by side in the periphery of the egg, and it may be that the latter is developing from the former. As a rule the mitochondria are inactive and seem to play no role in vitellogenesis. Russo in 1910 (quoted in Cowdry's book, **9**) expressed the opinion that 'in animals which had been starved, the mitochondria become vague and even disappear'. As in captivity snakes hardly get any food and are virtually starved prior to being killed, Russo's view may afford an explanation of the feeble development of mitochondria in these snakes.

The oocytes examined were not characterized by any prolific development of yolk, fatty or albuminous, though both kinds were observed. Fatty yolk is elaborated in some manner from the cytoplasm. This is inferred from the fact that in their early origin both Golgi and fatty-yolk bodies arise from the same area in the cytoplasm. Secondly, in pure turpentine, the fatty-yolk body disappears and the Golgi bodies surrounding it are left around a vacuolar area (fig. 5, Pl. 17). Thirdly, it is likely that the swollen Golgi bodies contribute their material to the formation of these bodies as their disappearance synchronizes with the active elaboration of fatty yolk.

A number of authors, Nath in Spider (**31**) and Culex (**32**), King in Oniscus (**22**), Gresson in Tenthredinids (**16**) and in Periplaneta (**17**), Nath and Mohan in the Cockroach (**35**), Nath and Husain in Scolopendra (**33**), Nath and Mehta in Luciola (**34**), have emphasized the Golgi origin of fatty yolk where one of the methods of development has been the deposition of free fat (often not miscible with the general cytoplasm) inside a Golgi vacuole. Somewhat similar, though not identical, conditions have been observed in Indian tortoises by the present writer (**3**) and in some amphibians by Narain (**29**). In the Ophidian oocyte fatty yolk is formed not by the deposition of fatty material inside a Golgi element, but in specialized areas of cytoplasm surrounded by a large number of minute Golgi bodies, the latter contributing their substance later to the formation of the finished fatty-yolk body.

In these snakes, fatty yolk is different from fat, and is in all probability a chemical compound having a concentration more lipoidal than fatty. This is inferred from two facts: (1) the fatty-yolk bodies are very poorly osmiophilic in comparison to these structures in other vertebrates. (2) They take a long time to become dissolved in fat solvents, such as xylol and pure turpentine. In the elaboration of a fatty-yolk body, it is likely that the lipoidal material is contributed by the Golgi bodies, specially those which become swollen, and the fat which is not present in any great concentration is built up from cytoplasm. Fat alone has been described to arise de novo from the cytoplasm in a number of animals—in Carcinus by Harvey (**18**) (in which the author makes no distinction between fatty yolk and fat), in Lygia by Steope (**40**), and in Discoglossus by Hibbard (**21**). The process in snakes may be said to go a step farther, inasmuch as it also derives material from the Golgi bodies, and forms fatty yolk as distinct from fat. Saguchi (**39**), in describing fatty-yolk formation in Amphibia, describes two successive phases in the deposition of fat which lead to the formation of the fatty yolk and recognizes fat as only one of the components of the fatty-yolk body.

The mode of formation of albuminous yolk-bodies cannot be described with certainty in Zamenis mucosus. They arise in the central cytoplasm and spread cortexwards. In other types they definitely arise in the cortex in close association with mitochondria and possibly under their influence. In the case of Amphibia, Saguchi (**39**) mentions the possibility of mitochondria, Golgi bodies, and the secondary deposit of fat globules participating in the formation of albuminous yolk, but denies any of these structures transforming directly into the latter body.

No nucleolar extrusions were noticed and their part in yolk formation, if any, is not known.

SUMMARY.

1. Golgi bodies are juxtanuclear in the early stages after which they migrate towards the cortex, getting finally dispersed in the cytoplasm. Some of the elements in the early stages swell up and become round; this stage is followed by the formation of fatty-yolk bodies. In general the individual Golgi elements have a lightly staining centre surrounded by a heavily

impregnated rim. Sometimes they also appear as crescents in section. These bodies are also present in the theca and the follicle cells of the oocyte and periodically 'infiltrate' inwards into the cortical region.

2. Mitochondria are feebly developed, and whenever met with are granular in early stages and dust-like and more peripheral in advanced oocytes.

3. Fatty yolk is short-lived and is formed in the cytoplasm under the influence of a number of Golgi bodies.

4. Albuminous yolk appears late in the development of the oocyte. It arises in the cytoplasm, sometimes in vesicles and sometimes in association with mitochondria in the peripheral region.

5. The four species of snake examined, Zamenis mucosus, Gongylophis conicus, Tropidonotus stolatus, and Tropidonotus piscator, are not very dissimilar with regard to the origin and behaviour of their cytoplasmic inclusions.

ADDENDUM.

Since the above was written the present writer has come across a paper by Beams and King, dealing with the cytoplasmic structures in the ganglion cells of certain Orthoptera, which he missed seeing before. This paper has a bearing on the present one inasmuch as it adds another instance in which the structure of the Golgi bodies, like that in snakes, is said to consist of an osmiophilic cortex and as osmiophobic medulla, which bodies sometimes also appear as crescents. The authors deny the existence of vacuome in the orthopteran ganglion cells, and consider it to be induced by a process of neutral red staining (**1***a*).

ACKNOWLEDGEMENTS.

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expressing my thanks to Dr. B. K. Das, Professor of Zoology in that University. I am also deeply grateful to Dr. R. A. R. Gresson, of the University of Edinburgh, for much helpful criticism and for supplying some references to the literature of the subject.

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EXPLANATION OF PLATE 17.

LETTERING.

GB, Golgi body in the oocyte; *gb*, Golgi body in the follicular epithelium cells; *GG*, Golgi groups or aggregated Golgi bodies; *Inf.G*, infiltrating Golgi body; *SG*, swollen Golgi bodies; *M*, mitochondria; *MC*, cloud of mitochondria; *FY*, fatty yolk; *AY*, albuminous yolk; *FE*, follicular epithelium; *ZR*, zona radiata; *Th*, theca; *N*, nucleus; *SM*, swollen mitochondria; *V*, vacuole.

Fig. 1.—Zamenis mucosus. Ludford preparation. Showing Golgi bodies and mitochondria aggregating round the nucleus. Also fatty yolk.

Fig. 2.—Zamenis mucosus. Mann-Kopsch preparation. Showing chiefly swollen Golgi bodies and mitochondria.

Fig. 3.—Zamenis mucosus. Ludford preparation. Showing fatty yolk formation.

Fig. 4.—Zamenis mucosus. Champy-Kull stain, after Champy fixation. Showing Golgi groups, albuminous yolk, mitochondrial cloud, &c.

Fig. 5.—Zamenis mucosus. Ludford preparation, treated with pure turpentine for over forty-eight hours, showing minute Golgi bodies concentrating round areas of decolorised fatty yolk *FY*.

Fig. 6.—Zamenis mucosus. A small central portion of the oocyte showing albuminous yolk. Champy-Kull stain after Ludford preparation.

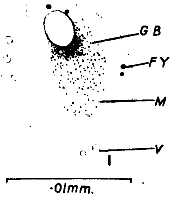
Fig. 7.—Gongylophis conicus. Ludford preparation, showing Golgi bodies in very early oocytes.

Fig. 8.—Gongylophis conicus. Ludford preparation stained with acid fuchsin and picric acid. Showing Golgi groups and the infiltration of the golgi bodies.

Fig. 9.—Tropidonotus stolatus. Mann-Kopsch preparation. Showing Golgi bodies and fatty yolk.

Fig. 10.—Tropidonotus stolatus. F.w.a. preparation. Champy-Kull stain. Showing Golgi groups, mitochondrial cloud, albuminous yolk, and Golgi bodies infiltering through zona radiata.

Fig. 11.—Tropidonotus piscator. Mann-Kopsch preparation. Showing Golgi bodies and mitochondria in an early oocyte.



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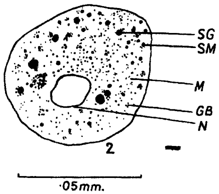

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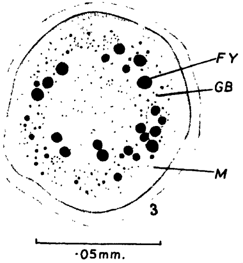

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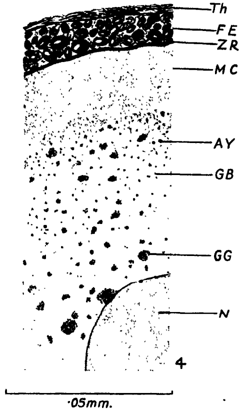

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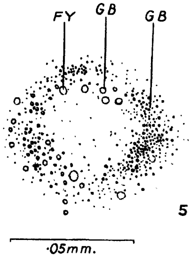

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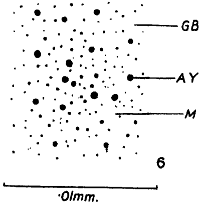

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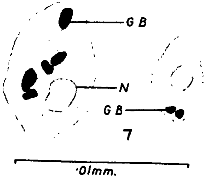

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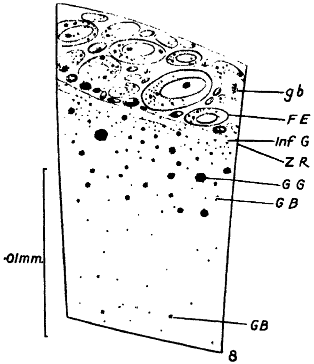

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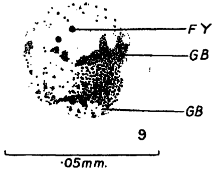

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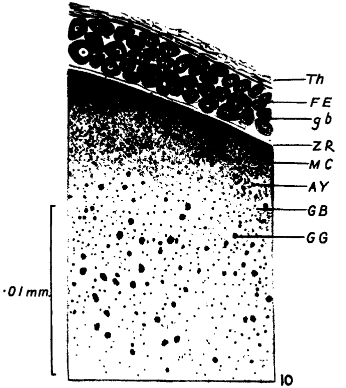

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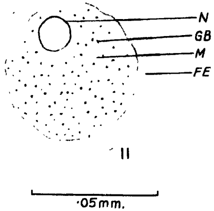

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K. B. Lal, del.

On a new Prorhynchid Turbellarian from Tasmania.

By

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With Plate 18 and 2 Text-figures.

INTRODUCTION.

DURING Easter 1932, while in camp with the Tasmanian Field Naturalists' Club at National Park, I had the opportunity of making a small collection of Turbellaria from the Mt. Field Plateau. This tableland lies about 3,400 feet above sea-level and forms part of the Central Plateau of Tasmania. The area consists largely of diabase and exhibits a glacial topography, which was probably developed during the Pleistocene period (Lewis, 1921, p. 21). It contains many examples of moraines, erratics, cirques, and glacial lakes. Here and there are small moors covered with a thick peaty soil, which is wet throughout the year. During the winter the whole area is often covered with snow.

The Turbellaria collected at this locality included six specimens of a new Prorhynchid, for which the name Prorhynchus tasmaniensis is proposed. The specimens were found in temporary pools, formed by rain, along the track from Lake Fenton to Wombat Moor. The European species of Prorhynchus appear to live in similar localities and under much the same alpine conditions. Steinboeck (1924, p. 241) states: 'Pr. hastatus und alpinus leben anscheinend nur im Bach- und Quellmoos unserer Gebirge. Der höchste Fundort von Pr. hastatus und alpinus . . . liegt auf der Gleinalpe 1900 m. hoch, von Pr. stagnalis ist es ein Hochmoor auf der Koralpe, in 1442 m. Höhe.'

Prorhynchus putealis Haswell and Prorhynchus haswelli Steinboeck and Reisinger occur in deep wells in

Canterbury, New Zealand. These two species appear to be the only members of the genus recorded from this zoogeographical region and also from the rest of the southern hemisphere.

Prorhynchus tasmaniensis sp. nov.

External Features.

When placed in water and allowed to move freely, two of the largest specimens measured 71·0 mm. long by 2·0 mm. wide. After fixation they measured 42·0 mm. long by 4·0 mm. wide. Bresslau (1928, p. 78) states that Prorhynchus putealis Haswell is the largest of the hitherto known Alloeocoela, its length being 25·0 mm. In life the Tasmanian species has a white, semi-translucent appearance. The intestine, which is often full of coloured food-particles, shows faintly through the body-wall, but the body itself is quite devoid of pigment. There are no eyes. In transverse section the living worm is strongly convex above and slightly convex below. The sides are produced into thin longitudinal flanges, which extend from the anterior to the posterior end of the body. These flanges are applied to the surface over which the animal is moving. They have a suctorial action which enables the Prorhynchid to cling to objects in the water. In preserved specimens the lateral flanges are very much reduced and the ventral surface of the body becomes strongly convex, even more so than the dorsal surface. The mouth is a wide oval aperture at the anterior end of the body. On each side of the mouth the body is produced into an antero-lateral angle.

The female genital pore lies in the middle line on the ventral surface and is only one-eighth of the body-length from the anterior end. The course of the convoluted germo-vitellarium may be clearly seen through the ventral wall of the body in the living animal. The two excretory pores are situated one on each side of the female genital pore and almost in line with it. They are three-eighths of the body-width apart. The openings of two ciliated pits occur on the ventral surface near the antero-lateral angles. The whole surface of the animal is clothed with cilia.

Internal Morphology.

For studying the internal anatomy the specimens were fixed in Gilson's Mixture (Lee, 1924, p. 47), which gave very satisfactory results. Fixation was followed by embedding in paraffin according to Mayer's method. Longitudinal and transverse serial sections were cut at a thickness of 6–8 μ. The sections were stained with Ehrlich's acid-haematoxylin and counterstained with eosin.

Body-wall and Parenchyma.

As in other Prorhynchidae the epidermis is an 'eingesenktes Epithel'. The nuclei of the epithelial cells are oval and have a long diameter equal to 15 μ. They have sunk into the parenchyma below the muscular layers. Between the epithelial cells and immediately below the surface of the body are numerous clear, vacuole-like glands, the secretion of which is not stained by either haematoxylin or eosin. Below these clear spaces is a distinct basal membrane, almost 3 μ in thickness.

The muscularis closely resembles that of Prorhynchus putealis Haswell as described by Steinboeck and Reisinger (1924, p. 444), but is even more strongly developed (fig. 1, Pl. 18). Below the basal membrane is a layer of circular muscles consisting of about six strata of fibres. Subjacent to these outer circular muscles is a layer of outer longitudinal muscles arranged in bundles of about twelve fibres. Below these we find the inner circular layer consisting of ten to twelve strata. Between this layer and the body parenchyma are the inner longitudinal muscles arranged in bundles of sixty to ninety fibres. The fibres vary in diameter from 3 to 9 μ. Here and there in the muscular layers are epithelial nuclei which have failed to sink into the parenchyma.

Prorhynchus tasmaniensis possesses two kinds of sunken integumentary glands, which, according to the reaction of their secretions toward stains, may be referred to as erythrophilous and cyanophilous glands respectively. The erythrophilous glands are unicellular. Each is ampulliform in shape and opens on the surface by a long neck, which passes through the muscular layers, and has a diameter of about 12 μ.

The basal portion of the gland is almost spherical and about 39 micra in diameter. It has sunk into the parenchyma. The cyanophilous glands are also in the parenchyma. They are smaller, more numerous, and very irregular in shape, sometimes being tubular, at other times lobed, branched, or tree-like. They open on the surface by fine ducts which frequently ramify as they pass through the muscular layers. The secretion of the erythrophilous glands is very dense, while that of the cyanophilous glands usually has an alveolar or froth-like appearance. Both kinds of glands are distributed throughout the body, but seem to be more numerous along the sides. As in other members of the genus rhabdites are absent.

Of the parenchyma there is nothing new to be said. It resembles that of Prorhynchus putealis Haswell.

Digestive System.

The mouth is an oval aperture at the anterior end of the body. It leads into a cylindrical pharynx, which is a pharynx variabilis intextus as in other Prorhynchidae. The wall of the pharynx is very thick and muscular. Passing outwards from the epithelium which lines the lumen of the pharynx, we have first a thick layer consisting of about 230 bundles of longitudinal muscles. Each bundle is made up of about sixty fibres. Outside this layer is a much thicker layer, the inner circular muscles. This is followed by a zone containing glands. External to the glandular zone is a second layer of circular muscles made up of only five or six strata, and finally a thin layer of longitudinal muscles. Extending from this outer layer of longitudinal muscles through the intervening layers to the inner longitudinal muscles is a large number of radial muscles. In addition to these a number of diagonal fibres pass from the inner longitudinal muscles through the outer layers and body parenchyma to the body-wall. These diagonal fibres correspond to the arched fibres, which in Prorhynchus putealis are said to extend from 'the external circular layer to the deeper part of the internal circular' in the pharynx (Haswell, 1898, p. 635).

The anterior end of the pharynx is much folded and capable of slight protrusion through the mouth. The length of the pharynx is about 5·0 mm. and its width 1·1 mm. in the adult animal. Its posterior end projects slightly into the lumen of the intestine. The latter organ is of large relative size. It occupies nearly seven-ninths of the total length of the body and extends almost to the posterior end. At intervals the lumen of the intestine is invaded by ingrowths from its wall and the surrounding tissue. About fifty such ingrowths occur. Dorsally and laterally these ingrowths are often occupied by the follicles of the testis, whilst ventrally they correspond with the windings of the germo-vitellarium. The epithelium lining the intestine consists of the usual elongated cells, which grow inwards to form a digestive syncytium, often completely filling the whole lumen. The anterior end of the intestine projects forward under the pharynx to form a short ventral diverticulum about 0·25 mm. in length (fig. 2, Pl. 18).

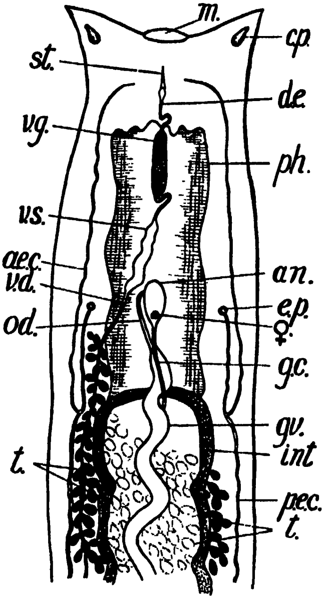
Excretory System.

As mentioned above, there are two excretory pores. They are situated on the ventral surface of the body, one on each side of the female genital aperture and almost in line with it. They are a short distance in front of the intestinal diverticulum. Each aperture leads into an elongated chamber, the wall of which is very thick and stains deeply with eosin. At its widest part the chamber is 46 micra in diameter. It passes upwards and outwards until above the ventral longitudinal nerve-cord. Here it gives rise to a longitudinal excretory canal, which follows a sinuous course above the nerve-cord towards the posterior end of the body. Slightly behind the level of the anterior end of the intestinal diverticulum this posterior excretory canal gives off from its dorsal side a somewhat narrower trunk, which bends sharply towards the front and runs almost to the anterior end of the body (Text-fig. 1). Here it bends towards the middle line and almost meets its fellow on the opposite side of the body.

In Prorhynchus putealis Haswell the posterior vessel bifurcates to form two branches, which run back side by side. In the Tasmanian species, however, no such bifurcation of the vessel occurs, and the excretory system approaches the typical form suggested by L. von Graff (1904, p. 2151).

Nervous System.

The brain consists of two large oval ganglia situated above and in front of the anterior end of the pharynx. Each ganglion measures 0·328 mm. long by 0·205 mm. wide. A stout commissure connects the two ganglia together. The four pairs of longitudinal nerve-cords described by Steinboeck and Reisinger as being present in Prorhynchus putealis Haswell are also present in the Tasmanian species. At regular intervals they are connected together by similar complete commissures. The



TEXT-FIG. 1.

Prorhynchus tasmaniensis sp. nov. Diagrammatic view of anterior quarter as seen from the ventral side. *a.e.c.*, anterior excretory canal; *an.*, antrum; *c.p.*, ciliated pit; *d.e.*, ductus ejaculatorius; *e.p.*, excretory pore; *g.c.*, genito-intestinal canal; *gv.*, germo-vitellarium; *int.*, intestine; *m.*, aperture of mouth; ♀, female genital aperture; *od.*, oviduct; *p.e.c.*, posterior excretory canal; *ph.*, pharynx; *st.*, stiletto; *t.*, testicular follicles (dorsal follicles in dotted outline); *v.d.*, vas deferens; *v.g.*, vesicula granulorum; *v.s.*, vesicula seminalis.

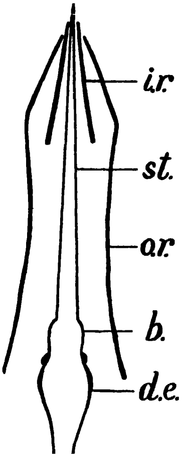
nervous system resembles that of Geocentrophora baltica (Kennel) and other Prorhynchidae so closely that there is nothing new to be mentioned.

Reproductive System.

It is in this system that we find the most marked differences between the Tasmanian form and other members of the genus Prorhynchus.

The male aperture is situated below and in front of the anterior end of the pharynx. It opens into the mouth. Inside the male aperture is the stiletto, 0·242 mm. long. Surrounding the stiletto is a conical mantle of longitudinal muscles, embedded in the inner surface of which are six chitinous rods. Each rod is about 0·123 mm. long, and the six of them are placed longitudinally at equal intervals round the stiletto. No dimensions have been given for the corresponding structures in Prorhynchus putealis Haswell, but in their diagram of the male apparatus of Prorhynchus stagnalis M. Schultze, Steinboeck, and Reisinger (1924, p. 447) show these rods, in the latter species, to be slightly longer than the stiletto. In the Tasmanian form the rods are only about half the length of the stiletto. Outside the mantle containing the six rods is another conical muscular mantle containing ten rods, each of which is 0·261 mm. in length (fig. 3, Pl. 18). The arrangement of the muscles is as in Prorhynchus stagnalis, M. Schultze. The stiletto is straight and needle-like. Its opening is just behind the point. The ten large rods in the outer mantle are curved in the manner shown in Text-fig. 2. From the stiletto a short winding ductus ejaculatorius leads back to a vesicula granulorum, which is a tubular sac, about 1·6 mm. long, lying in the middle line below the pharynx. Its lumen is about 0·122 mm. in diameter and its strong muscular wall 0·092 mm. in thickness. At its posterior end it narrows suddenly and passes into a coiled duct leading to the vesicula seminalis. This is a wide sac extending back in a sinuous course to the level of the excretory pores. The inner wall of the vesicula seminalis is somewhat folded and its epithelium is composed of cells with very large rounded nuclei. The folding of the wall may be due to the fact that the vesicle is almost empty. The vas deferens is a narrow duct which passes back on the right of the oviduct and finally runs up on the right side to enter the testis, from which it receives a number of small vessels (Text-fig. 1).

The testis is remarkable for its size and position. Benham (1901, p. 21) quotes Haswell as stating: 'that in Prorhynchus sp. the male organs are only on the right side, and the female only on the left side, unpaired in each case'. The paper to which Benham refers is that in which Haswell describes Prorhynchus putealis (cf. Haswell, 1898). However, I can find no such statement in this paper. In fact, on p. 640 Haswell makes the opposite assertion, namely, that the male organs are on the left side and the female on the right side. This position of the reproductive organs has recently been confirmed by Steinboeck (1924, p. 236). It should be noted, however, that Haswell's diagram (fig. 1, Pl. 18, Haswell, 1898) is obviously not in



TEXT-FIG. 2.

Prorhynchus tasmaniensis sp. nov. Diagram of a sagittal section through stiletto and surrounding chitinous rods. *b.*, enlarged base of stiletto; *d.e.*, dilated end of the ductus ejaculatorius; *i.r.*, a chitinous rod of the inner mantle; *o.r.*, a chitinous rod of the outer mantle; *st.* stiletto.

accordance with his own statement. The figure represents a ventral view of Prorhynchus putealis and shows the male organs on the animal's right side and the female organs on its left side. This error on the part of Haswell may have misled Benham.

Assuming, then, that Haswell's diagram is at fault and that his written statement, confirmed by Steinboeck, is correct, we have in Prorhynchus tasmaniensis the only known case of the testis being on the right side in a member of this genus. Not only does the testis lie on the right side, but its follicles extend over the dorsal side of the intestine on to the left side of the body. The anterior follicles are on the right side between the main excretory vessels and the pharynx. They are close behind the level of the excretory pores. The posterior follicles are just in front of the posterior end of the intestine. The vas deferens is unpaired and confined to the right side. It may be traced among the follicles for almost the whole length of the testis. The follicles on the right side communicate directly with the vas deferens. Those on the left seem to communicate with the dorsal follicles and these with the vas deferens. The testis is not bilaterally symmetrical, the number of follicles on the right side being greater than on the left side. The follicles are oval in shape and vary in length from 0·315 mm. to 0·344 mm. When the intestine is distended with food the follicles are often forced to occupy the ingrowths of the intestinal wall (fig. 4, Pl. 18).

In some of the testicular follicles there are sperm-like bodies of large size. They are quite unlike the normal sperms found in the vas deferens and other parts of the male duct (fig. 5, Pl. 18). Haswell seems to have noticed similar bodies in an immature specimen of Prorhynchus putealis, for he says (1898, p. 641) that he observed in the testis and its associated ducts 'a number of cells of peculiar character, having the appearance of malformed sperms of various stages'. If they are malformed sperms it is remarkable that they should appear in two different species.

The female reproductive apparatus is a germo-vitellarium. It occupies a median longitudinal position below the intestine and extends back in a sinuous course from the posterior end of the pharynx almost to the end of the intestine. Anteriorly it passes into a short oviduct, which lies in the mid-line ventral to the pharynx. This runs forward to open dorso-laterally into the right side of the antrum a short distance behind its anterior end. Steinboeck (1924, p. 235) states: 'Bei Prorhynchus stagnalis und putealis zieht der Ovidukt von rückwärts über (oder neben) dem Antrum hinweg und mündet von vorne und oben in dasselbe ein.' The antrum is a fusiform sac about 0·903 mm. long. It is lined with an epithelium of elongated cells, each of which has a large oval nucleus near its base. Near its posterior end the antrum opens by means of the female genital aperture on to the ventral surface of the body. As mentioned above, the female genital pore is only one-eighth of the body-length from the anterior end. In this respect Prorhynchus tasmaniensis differs from other members of the genus. Steinboeck (1924, p. 242) in defining his new group, Alloeocoela typhlocoela, states: 'Weibliche Geschlechtsöffnung im zweiten Körperdrittel.' This will need amending slightly if the Tasmanian species is to be included.

From the posterior end of the antrum a genito-intestinal canal runs backward below the oviduct and then upwards to open into the intestine at the level of the posterior end of the pharynx. The mode of opening into the intestine is interesting. The genito-intestinal duct passes through one of the ingrowths of the intestinal wall. This acts as a valve and prevents the contents of the intestine gaining access to the duct. The lumen of the duct is small and it is lined with ciliated epithelium throughout its whole length.

In mature specimens the oviduct, as well as the anterior part of the germo-vitellarium, is filled with yolk, embedded in which are a few large ova some distance apart. Scattered through the outer edge of the yolk are a number of small vitelline cells. Each ovum is an oval cell about 0·215 mm. long by 0·138 mm. wide. It contains a large nucleus 0·076 mm. long by 0·0614 mm. wide. Within the nucleus is a spherical nucleolus, 0·0307 mm. in diameter. Towards the posterior end of the body the germo-vitellarium becomes gradually narrower, the ova become smaller and more numerous, while the vitelline cells surround each ovum in a radial manner (fig. 6, Pl. 18).

SUMMARY.

1. Prorhynchus tasmaniensis sp. nov. is found under alpine conditions in Tasmania.

2. It is allied to Prorhynchus putealis Haswell, but exhibits the following differences: (*a*) Larger size. (*b*) Posterior excretory canal does not bifurcate. (*c*) Female genital pore almost in line with excretory pores and below pharynx. (*d*) Female genital pore only one-eighth of body-length from anterior end. (Difference from all other members of the genus.) (*e*) Germo-vitellarium in middle line ventral to intestine. (*f*) Testis follicular, extending from the right side over the intestine on to the left side. (Difference from all other members of the genus.)

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EXPLANATION OF PLATE 18.

Figs. 1–6. Microphotographs of transverse sections through Prorhynchus tasmaniensis sp. nov.

Fig. 1.—Through epidermis and subjacent structures. *cg*, cyanophilous gland; *eg*, erythrophilous gland; *icm*, inner circular muscles; *ilm*, inner longitudinal muscles; *ocm*, outer circular muscles; *olm*, outer longitudinal muscles. ×200.

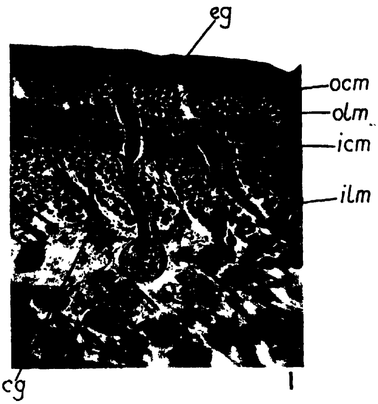
Fig. 2.—Through body slightly in front of posterior end of pharynx. *ev*, posterior excretory vessels; *gc*, genito-intestinal canal; *idm*, intestinal diverticulum; *od*, oviduct; *ph*, pharynx; *t*, follicles of testis; *vd*, vas deferens. ×17.

Fig. 3.—Through anterior end of stiletto and surrounding tissues. *ir*, inner ring of six chitinous rods; *or*, outer ring of ten chitinous rods; *st*, stiletto. ×350.

Fig. 4.—Through body slightly in front of posterior end of intestine. *gv*, germo-vitallarium; *int*, intestine; *t*, follicles of testis. ×20.

Fig. 5.—Through part of a testicular follicle. *ms*, abnormal sperm-like bodies. ×230.

Fig. 6.—Through the anterior part of the germo-vitellarium and adjacent structures. *gc*, genito-intestinal canal; *gv*, wall of germo-vitellarium; *n*, nucleus of ovum; *nls*, nucleolus; *o*, ovum; *vc*, vitelline cells. ×170.



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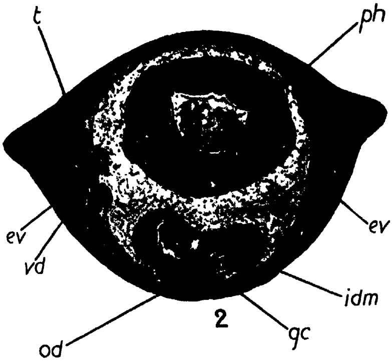

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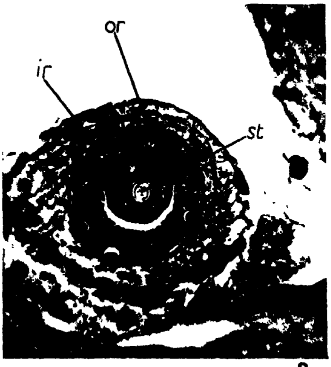

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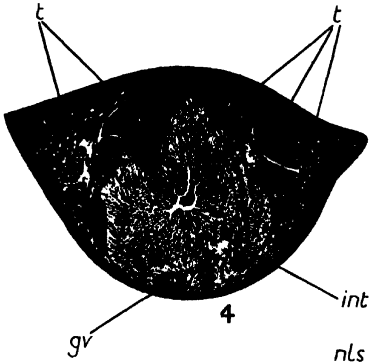

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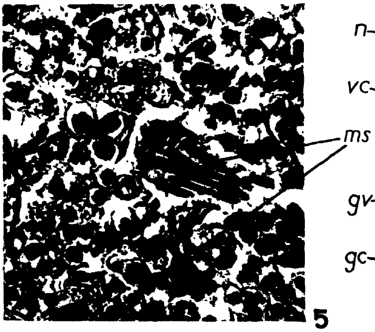

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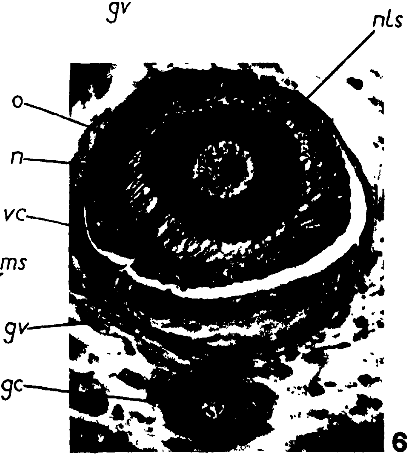

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V. V. Hickman, photo.

The Physiology of the Cuticle and of Ecdysis in Rhodnius prolixus (Triatomidae, Hemiptera); with special reference to the function of the oenocytes and of the dermal glands.

By

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From the London School of Hygiene and Tropical Medicine.

With 15 Text-figures.

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ECDYSIS in insects is the homologue of metamorphosis, and there can be small prospect of understanding the complex process of metamorphosis until the physiology of ecdysis has been adequately described. It is somewhat surprising, therefore, that so little attention should have been paid to the histological changes which take place during the moulting of non-metabolic insects; and the present work is an attempt to describe certain of these changes in the blood-sucking bug, Rhodnius prolixus.

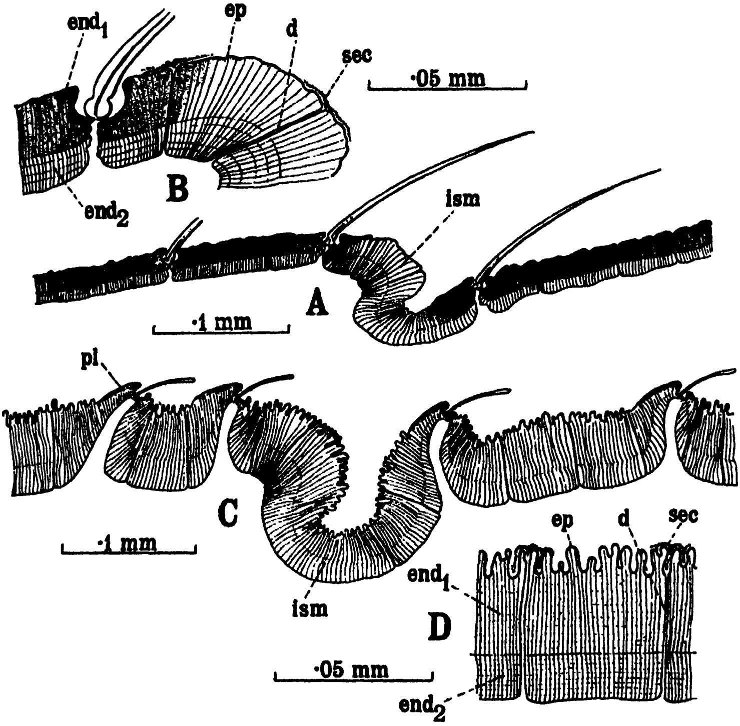
Most authors dealing with ecdysis, usually in caterpillars, date the commencement of the process from the time when the insect ceases to eat and to move about. But it is probable that the sequence of events which terminates in the casting of the old skin has been proceeding far longer than this, perhaps from the time of the previous moult, and that many of the essential stages have been overlooked. It so happens that in Rhodnius a single meal of blood suffices for each instar, and that ecdysis occurs at a definite interval after this meal, the whole process following a more or less constant time-table. This circumstance makes Rhodnius an admirable subject for such an investigation.

But the ingestion of a meal is followed by three processes: (i) the digestion, assimilation, and excretion of the ingested food (this occurs at all stages of life); (ii) growth, leading up to ecdysis (in the nymphal stages); and (iii) the maturation of the reproductive elements (in the adult and to a lesser degree in the fifth or last nymphal stage). In order to separate these processes, and to establish clearly which of the observed phenomena are truly connected with ecdysis, it has been necessary to study the changes which take place after a meal in the fourth nymphs, fifth nymphs, and adults of both sexes; and to observe, also, the changes which follow moulting when no meal is given.

For the present, the observations have been limited to the tergites and sternites of the abdomen; for these alone have proved sufficiently complex, and it is probable that the principles established apply equally to the remainder of the body.

1. STRUCTURE AND CHEMISTRY OF THE CUTICLE AND ITS BRISTLES.

Cuticle of the Adult.—Text-fig 1 A shows a longitudinal section of the sternites of the adult cut with the freezing microtome and mounted without staining. The cuticle consists of two primary layers: (i) a very thin epicuticle, and (ii) a relatively thick endocuticle.



TEXT-FIG. 1.

A. Longitudinal section of abdominal sternites of adult **Rhodnius**. B. Detail of same just in front of intersegmental membrane. C. Section of abdominal tergites of fifth nymph. D. Detail of same. All unstained. *d*, ducts of dermal glands; *end*$_1$, outer part of endocuticle, formed before moulting; *end*$_2$, inner part of endocuticle, formed after moulting; *ep*, epicuticle; *ism*, intersegmental membrane; *pl*, thickened plaque bearing bristle; *sec*, dried secretion from dermal glands.

The epicuticle is a uniform, refractile membrane less than 1 μ in thickness, either amber coloured or more or less impregnated with melanin. It corresponds with the 'Grenzlamelle' of German authors.

The endocuticle is divided by a more or less distinct horizontal line into an outer half (laid down before moulting) and an inner half (laid down after moulting). Its essential structure is most clearly seen at the intersegmental membranes (Text-fig. 1 B), where it is a thick colourless layer traversed by a great number of undulating vertical lines extending from the hypodermis to the epicuticle. These lines are the 'pore canals' of authors, the nature of which is a problem that has provoked much controversy. In the case of Rhodnius, however, there can be no doubt that they are fine canals containing protoplasmic filaments or fluid; for if the sections are allowed to dry on the slide and then mounted in Canada balsam, many of the pore canals contain air, appearing black by transmitted light and silvery-white by reflected light. (Similar observations were made by Hass (1916) on the cuticle of Crustacea.)

In sections fixed with Carnoy's or Bouin's fixatives and stained with haematoxylin and eosin, the pore canals are very indistinct, and they are apt to stain pink like the rest of the endocuticle. This pink staining has often been regarded (quite gratuitously) as evidence of chitinization; it is on these grounds that Holmgren (1902 *a*) and Plotnikow (1904) describe the 'pore canals' as solid filaments that have been transformed into chitin, and Berlese (1909) also adopts this view. On the other hand, if the whole cuticle after fixation is deeply stained with haematoxylin, the pore canals usually stain blue at least in the deepest parts of the cuticle.

The endocuticle shows also a horizontal striation, the laminae being most conspicuous in the inner half.

Such is the essential structure of the endocuticle as seen at the more flexible regions. But over the greater part of the abdomen the outer half of the endocuticle is impregnated with amber coloured material, more or less mixed with melanin, like the epicuticle. This rigid 'cuticulinized' layer (or 'sclerotized' layer, to use the term proposed by Ferris and Chamberlin (1928)) (Text-fig. 1 B, end_1) corresponds with the 'Emailschicht' of Biedermann (1903), the 'Lackschicht' of P. Schulze (1913), the

'Pigmentschicht' of Kremer (1920), and the 'exocuticula' of Campbell (1929); but since, at the intersegmental membranes and elsewhere, it is continuous with an entirely colourless endocuticle, and since it has the same basic structure as this, it seems preferable to regard it as an integral part of this layer. In these rigid areas the vertical striation (due to the pore canals) is very distinct, but the horizontal lamination seems wanting; in this it agrees with the structure of the dorsal plates of queen termites, as described by Ahrens (1930).

Chemically, also, the cuticle consists of two layers. Considering first the flexible type of cuticle seen at the intersegmental membranes: if the sections are heated in saturated caustic potash at 140° C. for five minutes, both epicuticle and endocuticle usually remain intact; and if the sections are now freed from alkali and treated with iodine in 1 per cent. sulphuric acid, the endocuticle shows an intense violet colour (chitosan test) while the epicuticle turns yellow. Chitin is therefore confined to the endocuticle. Similarly the biuret, xanthoproteic, and Millon's reactions are all intensely positive in the endocuticle but negative in the epicuticle. Thus protein also is limited to the endocuticle. Where the outer part of the endocuticle is impregnated with the amber coloured material, it still shows both the chitosan and protein reactions, although these may not be quite so intense; and in the core of the bristles likewise both these reactions are positive. More severe treatment with alkali (heating at 140–150° C. for an hour) removes entirely the amber coloured outer parts, leaving only the chitin in the endocuticle and in the small and limp remains of the bristles.

The converse experiment can be performed and the outer layers obtained free from chitin and protein by cautiously heating the sections in concentrated nitric acid. The colourless endocuticle swells up and becomes bright yellow, there is a vigorous evolution of gas,[1] the melanin is destroyed, and the protein and chitin of the endocuticle dissolve. At the intersegmental membranes nothing remains but the very thin epicuticle; elsewhere the amber coloured outer layer of the endocuticle also remains with the pore canals intact, and the bristles still retain their normal structure.

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[1] This gas is, doubtless, the product of oxidation by the nitric acid; it does not occur with sulphuric or hydrochloric acid.

When the inner part of the cuticle has been eliminated in this way, it is possible to study the properties of the outer part. This, as has been seen, is exceedingly resistant to strong nitric acid, although on prolonged heating it ultimately dissolves; and it is equally resistant to concentrated sulphuric acid in the cold and to hydrochloric acid. It gives an intense yellow colour with iodine, but does not stain with fat stains. It is insoluble in carbon disulphide and other fat solvents. The furfurol test is negative. On heating with a saturated solution of potassium chlorate in nitric acid, it melts into oily droplets (cerinic acid test). On warming in 40 per cent. caustic soda it fuses into an amorphous mass and dissolves. The biuret reaction is negative. On heating the dried membrane it partially fuses, and oily droplets appear which stain with Sudan III.

It is evident from these tests that the epicuticular substance is not a protein, carbohydrate, or a simple fat; its properties suggest that it might be a complex fatty or waxy substance or mixture. It forms the main constituent of the epicuticle or 'Grenzlamelle', which Kühnelt (1928) has shown to have the properties described above; but in addition it impregnates the protein and chitin of the outer part of the endocuticle. As Kühnelt points out, this material has the same function and the same chemical properties as the cuticle of plants; it is possibly allied chemically to 'cutin' or 'suberin'. These substances are themselves mixtures,[1] but it is convenient to have a name for them; similarly, it will be convenient to refer to the corresponding product of the insect cuticle as 'cuticulin'.

But just as the 'cutin' does not make up the whole cuticle of plants (van Wisselingh, 1895), so there are other substances besides 'cuticulin' in the outer parts of the cuticle of insects. For the nitric acid not only eliminates the melanin, but it removes the constituents which are responsible for the rigidity of the thicker parts. These are the constituents referred to by Schulze (1923) and Kühnelt (1928) as 'Inkrusten', and these authors give some evidence, derived from a study of the cuticle of beetles, for their being carbohydrates.

---

[1] Lee (1925) concludes that 'cutin is a complex mixture of fatty acids, both free and combined with alcohols, that have undergone condensation and oxidation; soaps of fatty acids and unsaponifiable material which probably contains some higher alcohols; resinous substances, and a compound giving tannin reactions'.

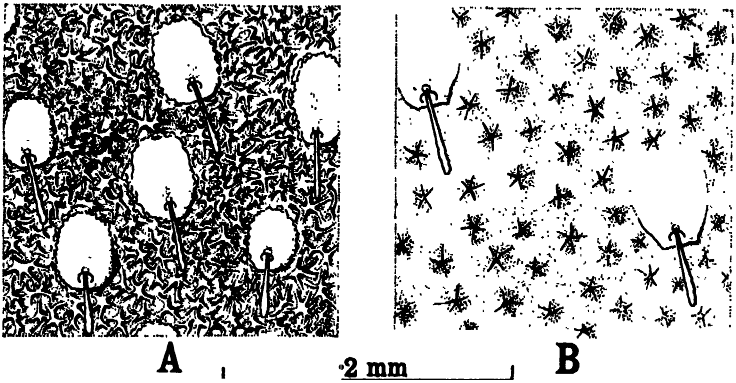
Thus the composition of the cuticle in Rhodnius may be summed up as follows:

Terminology adopted.		*Composition.*	*Synonyms.*	
Epicuticle		Cuticulin+ melanin, &c.	Grenzlamelle	Aussenlage or 'epidermis'
Endocuticle	Outer pigmented layer present only in rigid portions	Cuticulin+ melanin, &c.+ protein+chitin	Emailschicht Lackschicht Pigmentschicht Exocuticle	
	Inner colourless layer	Protein+chitin	Hauptlage, Innenlage or 'dermis'	

This agrees with the conception of Kühnelt (1928) except that he describes what is here called 'cuticulin' as being limited to the 'Grenzlamelle' (epicuticle), whereas in Rhodnius it is evident that the same material permeates also the outer layers of the endocuticle ('Pigmentschicht').

Finally, there is often mentioned in insects an outermost layer or 'Sekretschicht' supposedly produced by the dermal glands pouring out their secretion over the surface of the cuticle (Schulze, 1913; Stegemann, 1930). In Rhodnius this layer is discontinuous, and occurs only in little patches round the orifices of the dermal glands. As will be shown later, its presence is adventitious; it has no true relation to the structure of the cuticle and need not be referred to further here.

Cuticle of the Nymphs.—Text-fig. 1 c shows a longitudinal section of the tergites of the abdomen of the fifth nymph. The epicuticle again forms a very thin amber coloured membrane more or less impregnated with melanin. It is thrown into complicated folds except immediately around the bristles; here it is smoothed out to cover the prominent little plaques from which the bristles arise. The endocuticle is everywhere colourless and flexible, except around the bristles where the outer part is permeated with 'cuticulin' so as to form the rigid little plaques. It is again divided into an outer part (usually about two-thirds) laid down before moulting, and an inner part (one-third) laid down after; and it is traversed by 'pore canals' like those described in the adult. This verticle striation is very much more conspicuous (in sections from the freezing microtome) than the horizontal striation. In fresh material the latter is very difficult to make out, but it can generally be seen if the cut surface of the section is brought into focus. It is readily seen in sections cut with the freezing microtome, fixed in Carnoy, and stained with haematoxylin; and it is then apparent that the laminae are more widely spaced in the inner third. The chemistry of the two layers is the same as in the adult.

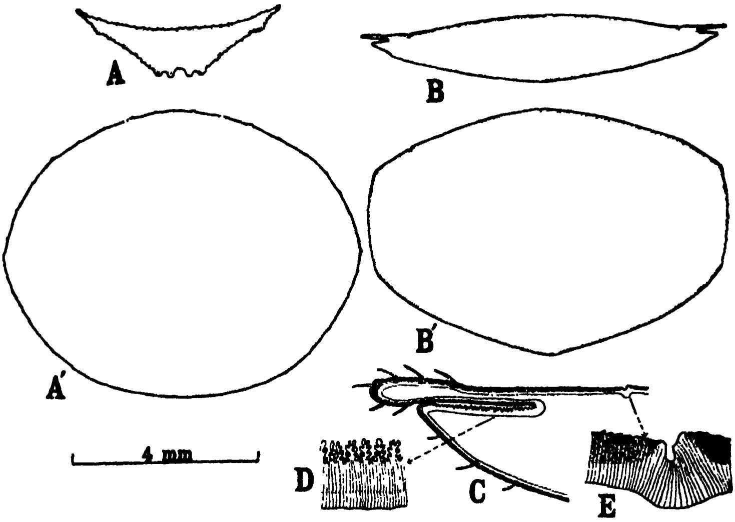


TEXT-FIG. 2.

A. Surface view of abdomen of unfed fifth nymph showing plaques bearing bristles, and stellate folds in epicuticle. B. Ditto, in fully fed fifth nymph.

Mechanism of Stretching.—The nymphal stages of Rhodnius ingest from six to twelve times their own weight of blood at a single meal (Buxton, 1930) and the adult may take three times its own weight. The abdomen must therefore be capable of enormous distension, which is accomplished in nymphs and adults in a totally different way.

In the nymphs, as we have seen, the endocuticle is flexible and free from 'cuticulin', except immediately around the bristles, and in the fasting insect the overlying epicuticle is thrown into deep stellate folds (Text-figs. 2 A and 3 A). When the abdomen is distended with blood, the endocuticle is stretched and attenuated (Text-fig. 3 A), while the stellate folds of the epicuticle become smoothed out (Text-fig. 2 B); it is this mechanism which provides for the enormous changes in volume. At the sides of the segments the tergites and sternites unite directly without the intervention of any elastic pleural membrane; but the unfolding of the intersegmental membranes contributes somewhat to the stretching process.



TEXT-FIG. 3.

A. Transverse section of abdomen of unfed fifth nymph. A′. Ditto, immediately after feeding. B. Transverse section of abdomen of unfed adult. B′. Ditto, immediately after feeding. C. Detail of lateral pleat in abdomen of unfed adult. D. Detail of extensible lower wall of this pleat. E. Detail of line of weakness towards margin of tergites.

In the adult, on the other hand, the outer part of the endocuticle is permeated with 'cuticulin' and is so rigid that it cannot be stretched. The chief provision for distension now lies at the sides of the abdomen, where there is a deep longitudinal fold or pleat (Text-fig. 3 B and C). The upper wall of this fold has a rigid cuticle like the rest of the abdomen; but in the lower wall the endocuticle is entirely soft and elastic and the epicuticle is excessively folded (Text-fig. 3 C and D). Consequently, when the adult is gorged with blood, not only are the intersegmental membranes and the lateral folds expanded, but the lower wall of each lateral fold becomes enormously stretched in the same manner as, but to an even greater degree than, the general cuticle of the nymphs (Text-fig. 3 B′). In addition to this, there is a line of weakness along either side of the tergites where the cutinization of the endocuticle is wanting (Text-fig. 3 C and E). Folding of the tergites occurs also along this line (Text-fig. 3 B′).

Ducts of Dermal Glands.—Besides the fine 'pore canals' which do not pierce the surface of the epicuticle, the cuticle is traversed by a great number of relatively large ducts connected with the dermal glands. In the nymphs these are fairly evenly distributed over the tergites and sternites of the abdomen, though they are more numerous in the immediate vicinity of the bristles. In the adult, they are evenly distributed over the sternites, but in the tergites they are very scanty in the central region (except over the last two segments) and are concentrated at the sides of the abdomen.

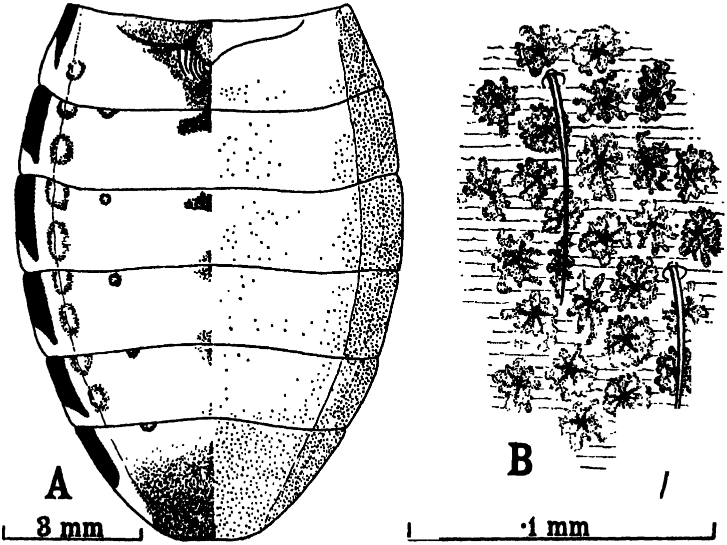
Their distribution is most readily demonstrated by placing the tergites or sternites (removed from a freshly killed insect) without fixation, in 0·05 per cent. methylene blue in Ringer's solution, and then fixing with ammonium picrate and acidified ammonium molybdate (Dogiel's method). This stains selectively the dried secretion around the orifices of the ducts, and renders them very conspicuous. Text-fig. 4 A shows the distribution of the ducts as demonstrated in this way; and Text-fig. 4 B the detailed appearance of the stained secretion. It is this secretion which constitutes the 'Sekretschicht' already mentioned (Text-fig. 1, *sec*).

The ducts of dermal glands are themselves sometimes referred to as 'pore canals' (the term is used in this sense, for example, by Henke (1924) in the case of Pyrrhocoris apterus) and it has sometimes been stated that they occur only in the pigmented areas. In unstained preparations this appears to be the case in Rhodnius, but only because the orifices of the ducts are difficult to make out in unpigmented places.

Text-fig. 4 A shows the lack of any true relation with the pigment, and this confirms the observations of Henke (1924) on Pyrrhocoris.

2. The Structure of the Epidermis.

Text-fig. 5 is a slightly schematized longitudinal section of the cuticle to show the various elements which compose the ectoderm or epidermis (hypodermis).



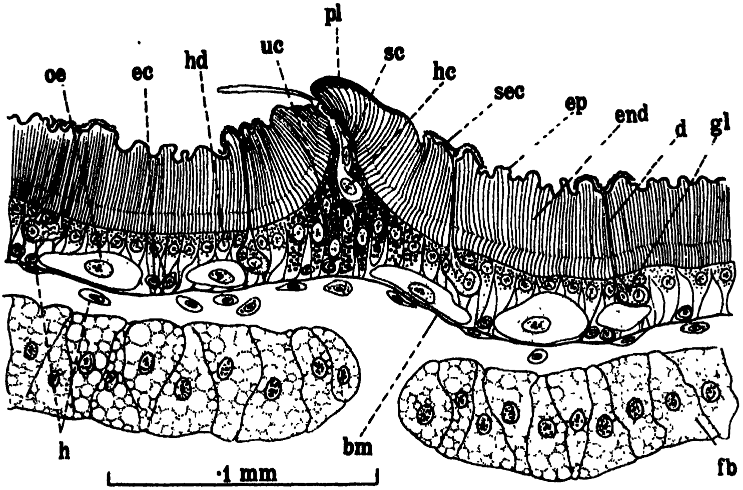
Text-fig. 4.

A. Tergites of adult showing on left the distribution of black pigment, and on right the distribution of ducts of dermal glands. B. Detail of dried secretion around orifices of these ducts stained with methylene blue.

The epidermal cells themselves (Text-fig. 5, *hd*) are usually conical with their pointed ends attached to the basement membrane, and their broad ends giving off very delicate processes which extend into the 'pore canals' of the cuticle. They contain numerous spheres of a brick-red pigment.

The epidermal cells beneath the plaques that bear the bristles differ from the others in being stuffed with spheres of uric acid[1] and containing very little of the red pigment. These will be referred to as urate cells (*uc*). In the centre of these are the large trichogen or hair-forming cell (*hc*) and the 'tormogen'[2] or socket-forming cell (*sc*).

Between the bases of the epidermal cells are the following structures: (i) the vestiges of dermal glands (*gl*) with their ducts opening through the cuticle; these are developed and functional only during moulting and will be described later; (ii) the oenocytes (*oe*), large cells with uniform eosinophil



TEXT-FIG. 5.

Longitudinal section of tergites of fourth nymph, somewhat schematic. *bm*, basement membrane; *d*, duct of dermal gland; *ec*, embryonic cells; *end*, endocuticle; *ep*, epicuticle; *fb*, fat body; *gl*, dermal gland; *h*, haematocytes; *hc*, hair-forming cell; *hd*, epidermal cells; *oe*, oenocytes; *pl*, plaque bearing bristle; *sc*, socket-forming cell; *sec*, dried secretion from dermal glands; *uc*, urate cells.

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[1] The chemical form of the uric acid, whether free or combined, is unknown. It gives a murexide test and Folin's colour reaction, and forms a precipitate with silver nitrate which quickly blackens in the light.

[2] Greek τόρμος: 'any hole or socket, in which a pin or peg is stuck' (Liddell and Scott).

cytoplasm; and (iii) undifferentiated or embryonic cells (*ec*), some of which, in due course, will give rise to the new dermal glands at the time of moulting, while others will give rise to the next generation of oenocytes.

The basement membrane (*bm*) is well developed and spreads as an unbroken sheet all over the body-wall, save where it is pierced at intervals by the muscles and by small tracheae, which break up into numerous tracheoles that ramify in all directions beneath the epidermis, adding their matrix cells to the elements mentioned above. Adhering to the lower surface of the basement membrane are haematocytes (*h*) of several kinds.

The fat body forms a rather slight fenestrated sheet below and often adherent to the basement membrane. It contains only a single type of cell, urate cells and oenocytes being restricted to the other side of the basement membrane.

3. GENERAL ACCOUNT OF THE PROCESS OF MOULTING.

Methods.—The morphological changes in the various elements mentioned in the last section have been studied throughout the fourth and fifth nymphal instars, and in adults of both sexes at all stages of digestion and reproduction.

Many of these changes are most readily observed in 'whole mounts' of the abdominal tergites and sternites. The dorsal and ventral walls of the abdomen have been fixed in Carnoy, freed from fat body and other deep structures, and stained with haematoxylin and eosin; or, for studying the uric acid cells and the pigment of the epidermis, they have been mounted unstained. A corresponding series of preparations has been embedded in paraffin and cut into serial longitudinal sections. Certain other experimental procedures for the elucidation of particular points will be described in the course of the argument.

Duration of Moult.—The fourth nymphs moult for the most part on the fifteenth and sixteenth days after feeding,[1] though some have moulted as early as the thirteenth day and

[1] The insects have been kept throughout at 24° C. in a damp incubator; all the times quoted refer to these conditions.

one as late as the twenty-first. The cause of these differences is obscure; they are certainly not due to differences in the interval since the previous moult, for batches of ten insects fed 1 week, 2 weeks, 3 weeks, 6 weeks, and 9 weeks after moulting all showed the same degree of variation, more than half the insects in each batch always moulting on the fifteenth and sixteenth days.

In the fifth nymphs, moulting has occurred from the twenty-fifth to the thirty-first day after feeding, but most individuals moult on the twenty-eighth day.

In the descriptions which follow, it will be convenient to allot a standard time of fifteen days to the fourth moult and twenty-eight days to the fifth, and to describe the sequence of events as though all the insects kept rigidly to the same time-table.

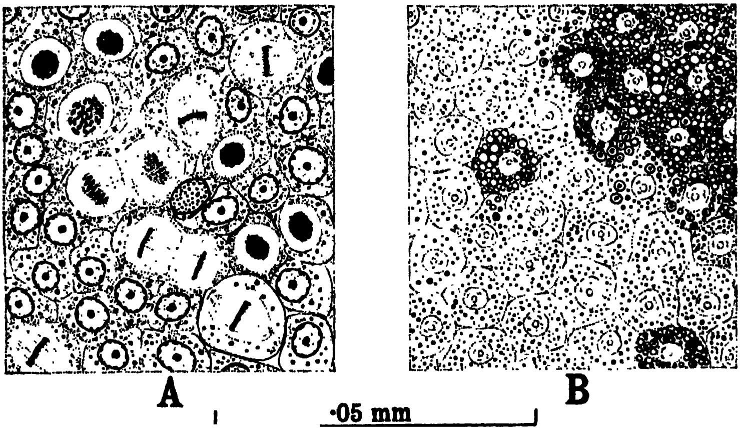
4. THE EPIDERMAL CELLS, URIC ACID CELLS, AND THE RED PIGMENT.

In the fourth nymph, within a day or so after feeding, the epidermal cells swell up and the granules of red pigment they contain begin to increase. By the fourth day, many of the cells along the intersegmental membranes have the nuclear chromatin shrunken away from the wall of the nucleus, and by the fifth day mitosis has begun. Multiplication is most intense along the intersegmental membranes, but by the sixth day mitotic figures can be seen all over the tergites and sternites (Text-fig. 6 A).

Now it is obvious that, in order to divide, the cell must detach itself from the cuticle, and it is highly probable that once it has become detached it must remain so. Hence, if division occurs in all the epidermal cells (and it is so widespread that this appears to be the case), then once it has taken place over the whole surface of the body, the insect will have freed itself from the old cuticle; it only remains for the new cuticle to be laid down and the old cuticle to be absorbed for moulting to be complete.

Conversely, it is clear that multiplication of the epidermal cells cannot take place once the new cuticle is laid down, and thus it is that the first appearance of the cuticle coincides with the cessation of mitosis at about the ninth day.

During this multiplication process, the red pigment continues to increase, reaching a maximum at the eighth or ninth day. The increase is most marked along the intersegmental membranes, where the cells become so densely crowded and later folded to form a triple layer. Exactly parallel with the pigment the uric acid also increases. The urate cells, as they become stuffed with the uric acid spheres, form opaque white spots beneath the bristles; and besides these cells there are others, scattered at intervals along the intersegmental membranes and along the margin of the abdomen where the tergites and sternites join, which also become charged with uric acid. Like the red pigment, the uric acid reaches a maximum at about the eighth day. Thereafter it begins to disappear, and by the time moulting takes place it is greatly reduced. The pigment follows the same course, but it diminishes more slowly.



TEXT-FIG. 6.

A. Epidermal cells on abdominal tergites of fourth nymph six days after feeding. B. Epidermal cells on abdominal tergites of fifth nymph nine days after feeding, showing uric acid spheres many of which have formed around granules of red pigment, shown black (unstained).

(Spheres of uric acid (or urate) were observed by Poisson (1925) in the epidermal cells of Notonecta in the nymphal stages, but no explanation of the phenomenon was suggested.)

What is the significance of this accumulation? The uric acid is certainly a waste product, but since it is the chief excretory substance removed from the blood by the Malpighian tubes (Wigglesworth, 1931), it seems more probable that the uric acid in the urate cells is produced locally, than that it is taken up from the blood. Now one of the chief constituents of the cuticle is chitin, a substance very poor in nitrogen, and this is produced by the insect from the highly nitrogenous blood protein on which it feeds. Consequently in the formation of chitin there must be a large production of nitrogenous waste, and it seems likely that this is the explanation of the appearance of uric acid in the epidermis during ecdysis.

It remains to be explained why the uric acid is confined to certain cells. Now it is very striking that it is deposited chiefly in the cells below the plaques bearing the bristles. These cells are crowded into deep pits, and are therefore much more protected from the circulating blood than the epidermal cells elsewhere; and it is possibly this circumstance, that the uric acid is produced more rapidly than it can be eliminated, which is responsible for its crystallization.[1] There are two other facts which lend colour to this hypothesis: (i) uric acid spheres appear also in other places sheltered from the blood, for example, along the intersegmental membranes, at the margin of the abdomen where the tergites and sternites unite, and in the mid-dorsal line where the heart is closely applied to the body-wall; (ii) as soon as the cells retract from the pits below the bristles, the uric acid begins to disappear; but soon the new pits are formed and uric acid persists in the depths of these until the next moult. On the other hand, in the formation of the adult tergites, in which these pits do not occur, the disappearance of the uric acid takes place very strikingly as soon as the new cuticle begins to form. By the time moulting occurs very little remains; most of this dissolves away during the next ten days, and none reappears after feeding in the adult stage.

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[1] On the other hand, uric acid spheres do not occur in the hair-forming and socket-forming cells which lie at the centre of the urate cells.

If the view put forward here is correct, the uric acid that appears in the epidermal cells is simply the product of their own metabolic activity and there is no reason to regard these cells as organs of excretion.

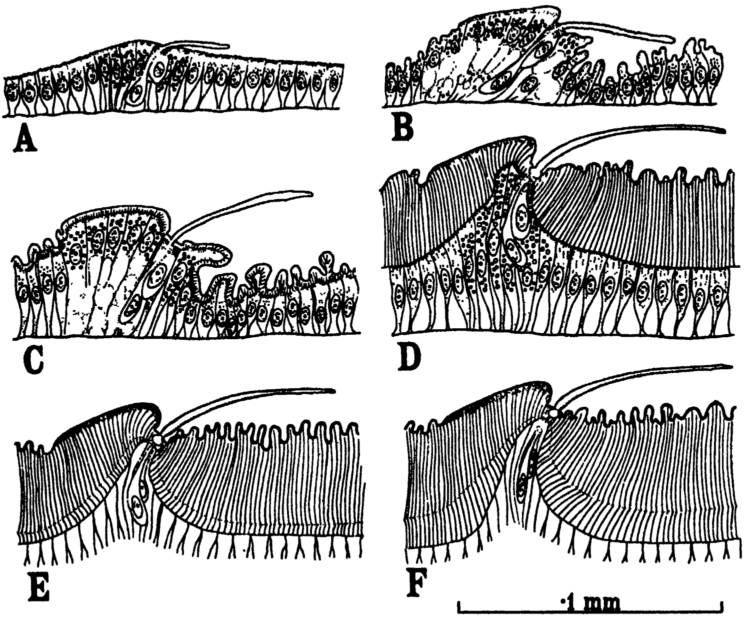
The red pigment of the epidermal cells increases in amount exactly parallel with the uric acid, and this suggests that it also is an excretory by-product of the synthetic activity of these cells. It is much more widespread than the uric acid, occurring in all the epidermal cells, but this may be due to its much greater insolubility. There is no sharp distinction between the cells that contain uric acid and those that contain pigment; many of the cells contain both, the red granules of pigment serving as nuclei upon which the uric acid crystallizes out (Text-fig. 6 B).

The fate of the pigment is probably the same as that of the uric acid—slow diffusion into the blood and excretion by the Malpighian tubes. It is an exceedingly insoluble substance, and this perhaps explains the slowness of its disappearance; but after passing a maximum it gradually decreases, and in the adult insect that has moulted some weeks before very little remains. Perhaps this is the source of the yellow pigment that occurs in the urine in Rhodnius (Wigglesworth, 1931), for the brick-red pigment and the yellow pigments of Hemiptera are closely related (Henke, 1924).

In the fifth nymph the sequence of events is precisely the same; mitoses occur from the sixth to the fifteenth day, and cease just before the new cuticle is laid down. The uric acid and red pigment reach a maximum round about the twelfth day. None of these changes take place after a meal in the adult; they are all clearly associated with moulting.

5. FORMATION OF THE NEW CUTICLE AND BRISTLES.

In the Fourth Nymph.—At the ninth day, when mitoses are almost at an end, the epidermal cells can readily be detached as a continuous layer from the old cuticle; and if this layer is examined in surface view, it is seen to be covered by an exceedingly delicate smooth membrane which in stained preparations shows a finely fibrillar structure. By the tenth day (Text-fig. 7 A) this membrane shows signs of crinkling, and by the eleventh day it is thrown into conspicuous stellate folds (Text-fig. 7 B). It therefore corresponds clearly with the epicuticle, but at this stage it stains with haematoxylin.



TEXT-FIG. 7.

Longitudinal sections showing formation of new cuticle during moulting of fourth nymph. A. Ten days after feeding. B. Eleven days. C. Twelve days. D. Just before moulting (fifteen days). E. Twenty-four hours after moulting (now fifth nymph). F. Four days after moulting.

It is interesting to consider the mechanism by which the folding of this membrane may be effected. The folding must be a spontaneous change occurring in the membrane itself, for the folds bear no relation to the arrangement of the underlying cells. The secretion of the cells evidently coagulates at first to form a smooth membrane, which then expands (perhaps by the imbibition of water from the moulting fluid outside it) and becomes folded, falling naturally into stellate folds (see Text-fig. 2 A) like those in the skin which appears at the surface of hot milk.

At this stage the cuticle contains no chitin, and it quickly dissolves on heating in saturated caustic potash. It gives a very weakly positive Millon's reaction, but on warming in the Millon's reagent, it persists for a time as a colourless membrane free from protein, and then dissolves. Although evidently the epicuticle, it has not yet acquired its resistant qualities.

The membrane that is going to form the plaques around the bristles does not become folded but remains smoothly adherent to the 'urate cells' beneath it. Then these cells develop large basal vacuoles which cause the cuticular surface to bulge outwards; in this way the raised plaques are moulded (Text-fig. 7 B and C).

At the same time the formative cell of the bristle, which lies at the centre of the group of urate cells, sends out a fibrillar prolongation which forms the basis of the new bristle (see p. 289).

As soon as the epicuticle is laid down, the formation of the endocuticle begins, and the chitosan test applied at the twelfth day reveals a delicate chitinous sheet moulded to the lower surface of the epicuticle. The endocuticle shows its vertically striated appearance almost from the outset. The exact mechanism of its formation is not known, but it must evidently be separated from the cells in fluid form around the protoplasmic filaments that occupy the 'pore canals', because there is no sign of any partitioning ('Felderung') of the endocuticle to correspond with the underlying epithelium, such as occurs in those cases where the cuticle arises by a transformation of the superficial layers of the epidermal cells. It can often be noticed that the granules of pigment in the epidermal cells arrange themselves in vertical lines (Text-fig. 9 C and D). This suggests that there are invisible filaments running through the cells, perhaps continuous with those in the 'pore canals'.

As Berlese (1909) points out, the formation of the chitinous parts of Arthropods is certainly accomplished in many different ways. A fluid secretion may condense into a solid sheet, as in the formation of the peritrophic membrane in many insects

(Vignon, 1901; Wigglesworth, 1930), or the chitin may be separated within the body of the cell itself, as in the formation of the cuticle of certain Crustacea (Vitzou, 1882) and in the development of cuticular hairs and bristles (see p. 289). The mechanism in Rhodnius seems to agree most closely with that described by Leydig (1864) and by Braun (1875) in Astacus, by Holmgren (1902 *a*) in the oviduct of Calliphora, and by Poisson (1924) in aquatic Hemiptera, which consists in the deposition of chitin in fluid or semi-fluid form around filiform outgrowths from the epidermal cells, which outgrowths later constitute the pore canals. As has often been pointed out before, this mechanism recalls the formation of dentine by the odontoblasts of vertebrates (Hass, 1916).

By the thirteenth day, when the endocuticle is about one-third its final thickness, the epicuticle and the bristles are becoming amber coloured; they are more resistant to solution in concentrated nitric acid than the endocuticle, but dissolve fairly easily on heating. The endocuticle contains its normal constituents: protein and chitin. At this stage it is very difficult to detect any horizontal striation of the endocuticle.

Text-fig. 7 D shows the appearance of the cuticle at the time of moulting, before the epicuticle has darkened. Twenty-four hours later (Text-fig. 7 E) the epicuticle has darkened and the inner layer of the endocuticle is appearing. By the end of four days (Text-fig. 7 F) the endocuticle is fully developed. It will be seen that as the pore canals cross the boundary between the inner and outer layers, they often change their direction slightly. This indicates that at the time of moulting the cuticle has been displaced somewhat in relation to the epidermal cells.

In the fifth nymph the sequence of events is the same. The non-chitinous epicuticle first appears at about the sixteenth day after feeding. It becomes slightly folded during the seventeenth and eighteenth days, and then the chitinous endocuticle is formed. At the time of moulting, the bristles are strongly 'cuticulinized' and amber coloured, but the impregnation of the outer part of the endocuticle with 'cuticulin' is incomplete, and this layer is still almost colourless. It darkens and hardens during the next day or so, while the inner half of the endocuticle is being deposited.

The most important conclusion from these observations is that the outermost layer, the epicuticle, is laid down first, and the endocuticle then formed below it; and that the impregnation of the cuticle with 'cuticulin' takes place progressively while the endocuticle is being laid down. This impregnation may possibly continue after moulting, or it may be that the 'cuticulin' already present merely undergoes oxidation when exposed to the air, becoming more resistant to reagents, like a 'drying oil' or varnish.

This mode of formation of the epicuticle agrees with that described in Leptinotarsa by Tower (1906), who calls the epicuticle 'primary cuticula' and the endocuticle 'secondary cuticula'. It is contrary to the belief of Schulze (1913) deduced from a study of the elytra of beetles, that the 'Grenzlamelle' (epicuticle) is formed by the outpouring of the secretion of the dermal glands over the surface of the endocuticle; nor does it agree with the recent observations of Yonge (1932), who has shown that the outer layer of the cuticle of Decapod Crustacea is almost certainly formed by the dermal glands in this way.[1]

Formation of the Bristles.—The method of formation of the bristles agrees exactly with that described in Saturniid larvae by Haffer (1921) and in Hyponomeuta (Lep.) by Hufnagel (1918). Two cells are concerned in the formation of each bristle, a hair-forming cell ('Haarbildungszelle'), and a socket-forming cell ('Haarpfannenzelle'). Haffer describes how the hair-forming cell sends out a process which passes through the annular socket-forming cell 'as the finger is passed through a signet ring'. He describes and figures this process from sections, and it is similarly figured from the fourth nymph of Rhodnius in Text-fig. 7. But the aptness of the signet ring analogy is best appreciated when the process is studied in surface view. Text-fig. 8 shows several stages in the formation of the bristles on the sternites during the moulting of the fifth nymph, as seen in whole mounts.

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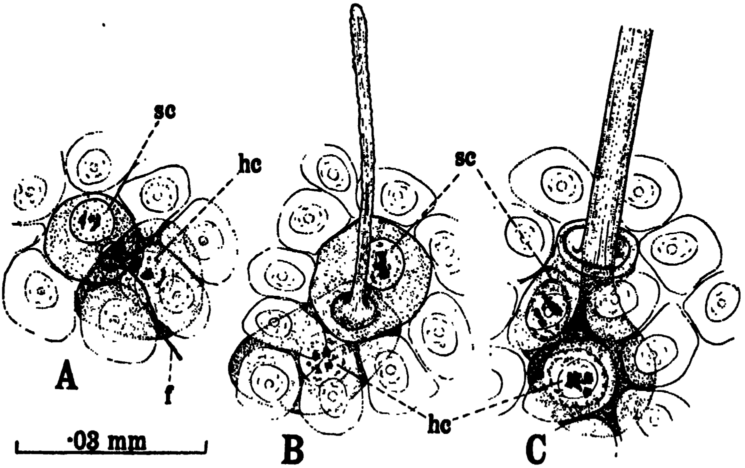
[1] It may be pointed out here that the outer layer of the cuticle in Decapod Crustacea as described by Yonge has very different properties from the epicuticle of insects: it stains with basic dyes, and with lipoid stains, and it readily imbibes water.

The outgrowth stains rather deeply at first and is covered by a delicate basophil membrane continuous with the newly formed epicuticle over the epidermal cells. When fully grown, the bristle gradually becomes eosinophil; finally, it becomes amber coloured and impregnated with 'cuticulin' and will not stain at all. A central canal always remains. This process demonstrates very clearly (i) the deposition of chitin within a cellular outgrowth, and (ii) the progressive impregnation of this chitin with 'cuticulin'.



TEXT-FIG. 8.

Formation of bristles in sternites (of adult) during moulting of fifth nymph. A. Thirteen days after feeding. B. Sixteen days. C. Eighteen days. *f*, basophil filament; *hc*, trichogen or hair-forming cell; *sc*, 'tormogen' or socket-forming cell.

Text-fig. 8 A shows a basophil filament (*f*) which lies immediately in front of the bristle. This represents the other end of the filament (*f*) shown in Text-fig. 10 A and comes from the corresponding bristle of the old cuticle. Judging from Haffer's account, this is perhaps the distal filament of the neurone which innervates the bristle, but the nerve supply of these structures has not been studied in Rhodnius. Apparently the same hair-forming and socket-forming cells persist from one moult to the next.

Pigmentation of the Cuticle.—The late progressive changes in the cuticle, described above, suggest that the epidermal cells, presumably by way of the pore-canals, can still exert an influence upon the epicuticle after the endocuticle is formed; and that this is so is proved by certain observations on the pigmentation of the cuticle. In one experiment, which has been several times repeated and confirmed, the old cuticle was stripped from the region of the fourth and fifth abdominal tergites in a fifth nymph forty-eight hours before moulting. The epicuticle was already fully formed; but instead of darkening within an hour or so after exposure to the air, as occurs in the insect moulting normally, the exposed area remained pink and soft for two days. The insect then moulted, and pigmentation at once began in all parts of the body.

In many parts of the cuticle, particularly in the nymphs, melanin is restricted to the epicuticle. We have already seen (p. 278) that the formation of melanin cannot be connected with the secretion of the dermal glands. The influence which induces pigmentation must therefore be exerted from within the body, through the substance of the epicuticle. Perhaps this is the function of the pore-canals—to enable the epidermal cells to effect enzymic changes in the epicuticle after moulting is complete.

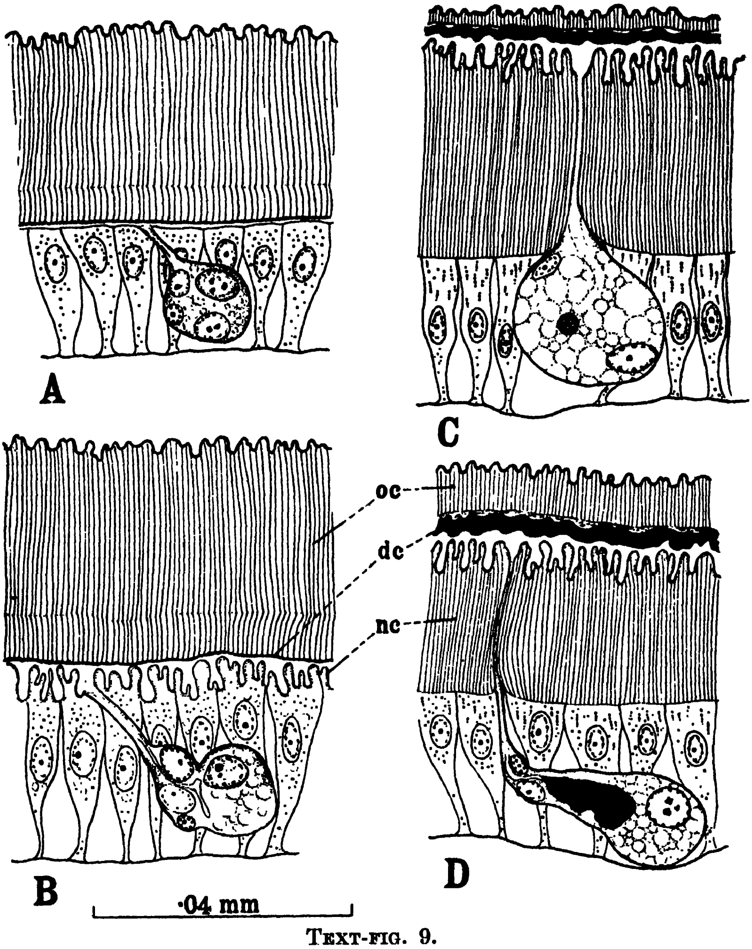
6. THE DERMAL GLANDS AND THE MOULTING FLUID.

The dermal glands are formed anew at each moult from undifferentiated or embryonic cells in the epidermis. They are functional only during moulting and then degenerate. They are absent in the adult.

In the fourth nymph they first become recognizable at about the eighth day after feeding as clusters of four cells, each of which has a nucleus of a distinct type which it is not necessary to describe in detail. Clearly each of these cells is destined to contribute a different element to the final gland. One becomes the secretory cell proper, one forms the intracellular duct of the secretory cell, one appears to be responsible for the part of the duct which passes through the epidermis and will later be included in the new cuticle, and perhaps the fourth supplies a delicate capsule for the whole structure. In the late stages of development the fourth cell often cannot be found.

Text-fig. 9 A shows the rudiments of a gland at the eighth day; the duct is being laid down, and it is already evident which cell is going to form the gland proper. By the eleventh day (Text-fig. 9 B) the gland-cell is becoming vacuolated and the duct is well developed. Up to this stage it is impossible to detect any certain differences between one gland and another, but in the later stages of moulting there are two very distinct types. Text-fig. 9 C and D shows these at the time of maximum activity shortly before moulting. In type 'A' (Text-fig. 9 C) the gland-cell is excessively vacuolated, the vacuoles being larger peripherally; the intracellular duct ends in a small round vesicle, which in some sections contains material staining blue with haematoxylin, and the whole structure lies close beneath the cuticle. In type 'B' (Text-fig. 9 D) the vacuolation of the gland-cell is not so extreme, there is a very large intracellular saccule distended with blue staining secretion, and the gland lies below the general level of the epidermal cells. The duct of type 'B' is narrower. Both types are evenly distributed all over the tergites and sternites, but there is always a group of three or four immediately round each bristle. Type 'B' is by far the more numerous.

In the fifth nymph the two types are distinguishable at a relatively much earlier stage. By the sixteenth day type 'A' is already recognizable (Text-fig. 10, *a*), but type 'B' is still at the stage of four scarcely differentiated cells (Text-fig. 10, *b*). The subsequent development of type 'A' is just like that in the fourth nymph, the vacuolation becoming more and more extreme so that the adjacent epidermal cells are forced aside; Text-fig. 10, *g* shows their appearance at the twenty-seventh day, shortly before moulting. Text-fig. 10, *b* to *f*, shows the subsequent development of type 'B', as seen in whole mounts. When fully formed (Text-fig. 10, *f*) the gland-cell is less vacuolated than in type 'A', but the intracellular duct is very similar,

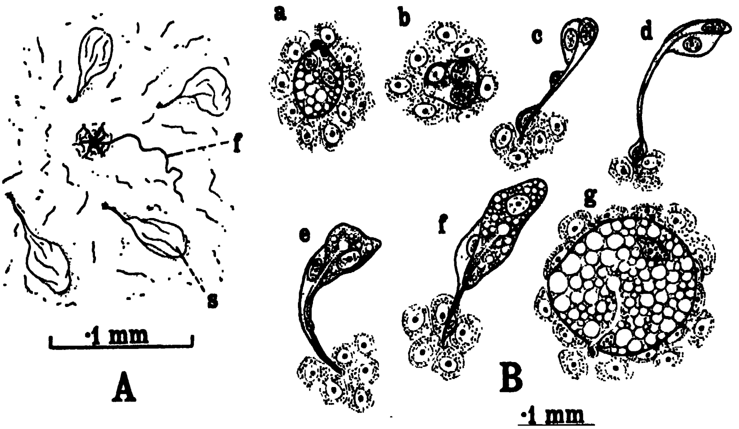


TEXT-FIG. 9.

Formation of dermal glands during moulting of fourth nymph; longitudinal sections. A. Eight days after feeding; cells separated from cuticle, new dermal gland forming. B. Eleven days after feeding; dermal gland becoming active; new epicuticle formed; digestion of old cuticle just beginning. C. Fourteen days after feeding; dermal gland type 'A' at height of activity; old cuticle almost entirely digested, the part in process of digestion appears as blue staining layer. D. Thirteen days after feeding; dermal gland type 'B' fully active, with its saccule containing basophil material. *oc*, old cuticle; *dc*, layer of old cuticle in process of digestion; *nc*, new cuticle.

ending in a small round vesicle and not a large saccule as it does in the fourth nymphs.

In the sternites of the fifth nymph (that is to say the sternites of the developing adult) both types are evenly distributed; but in the tergites, type 'A' occur chiefly over the more central regions, while type 'B' are concentrated in enormous numbers along the margins of the segments (Text-fig. 4 A).



TEXT-FIG. 10.

A. Innermost layer of old cuticle discarded during moulting of fourth nymph. *f*, filament from old bristle; *s*, lining of saccule of old dermal gland. B. Development of dermal glands during moulting of fifth nymph as seen in whole mounts. *a*, type 'A', sixteen days after feeding; *b*, type 'B', sixteen days after feeding; *c*, ditto, eighteen days; *d*, ditto, twenty days; *e*, ditto, twenty-four days; *f*, ditto, twenty-seven days; *g*, type 'A', twenty-seven days after feeding.

During moulting the cells composing the old moulting glands break down; their nuclei undergo chromatolysis, and chromatin spheres derived from them are set free among the epidermal cells. These will be mentioned again later (p. 308).

Function of the Dermal Glands.—Dermal glands associated with moulting occur in many groups of insects (Plotnikow, 1904). They were first recognized by Verson (1902) in the larva of the silkworm, and he ascribed to them two functions: (i) the production of an exuvial fluid which serves as a lubricant and facilitates the casting of the old skin, and (ii) the excretion of urates and oxalates vicariously on behalf of the Malpighian tubes, which are supposed to be temporarily out of action through a sort of physiological stoppage..

The excretory function of these organs can probably be dismissed, for it was shown by Plotnikow (1904) that the crystals which appear in the exuvial fluid are really derived from the Malpighian tubes, and have spread forwards from the anus beneath the old cuticle; an observation which has recently been confirmed by Shimizu (1931). There is no sign of any excretory substances in the moulting fluid of Rhodnius.

The origin of the moulting fluid from the dermal glands has been generally accepted by Plotnikow (1904), Tower (1906), Schulze (1912), &c.; on the other hand, v. Buddenbrock (1930, 1931), in the case of Lepidoptera, regards this fluid as a product of the epidermal cells (as also do some other authors, notably Blunck (1923) in Dytiscus), and gives some rather indirect evidence for the view that the glands of Verson are endocrine organs discharging into the blood of the larva a hormone which induces moulting.[1] In the case of Rhodnius this hypothesis is untenable, because moulting (as indicated by the mitosis and separation of the epidermal cells) has already begun by the fifth day after feeding in the fourth nymph and by the seventh day after feeding in the fifth nymph—long before the dermal glands are differentiated, far less fully active. Moreover, the ducts of the glands are so distinct that there is no reason to doubt that they are responsible for the secretion of the moulting fluid.

The chief question is the function of this fluid. The majority of authors regard it primarily as a lubricant to facilitate moulting. Yet it is a matter of observation that most insects are almost dry at the time the skin is cast, whereas if they are dissected a day or two before moulting, there is abundant fluid present. It is probable, therefore, that the main function of the moulting fluid is accomplished before ecdysis actually occurs.

Various authors (Plotnikow, 1904; Tower, 1906) have noted that the old cuticle is largely softened and absorbed during

[1] See also Hoop (1933).

moulting, and Tower suggests that perhaps an enzyme is concerned in this process; but the extent to which such absorption occurs does not seem to have been generally appreciated. It is illustrated by the following observations.

The abdominal tergites and sternites were dissected off ten fifth nymphs of Rhodnius twenty-four hours after feeding, freed from cellular tissue by rubbing the inner surfaces with cotton wool, dried in the air and weighed. The average weight was 3·7 mg. The corresponding parts were then dissected from the cast skins of ten fifth nymphs. They showed an average weight of 0·5 mg.; in other words, 86·5 per cent. of the abdominal cuticle has been digested and reabsorbed by the insect.

The course of this absorption is shown clearly in Text-fig. 9. It begins when the dermal glands become functional (at about the eleventh day in the fourth nymph and the twenty-first day in the fifth nymph) and it is most rapid in the last few days of moulting when these glands are at their zenith. Thus it is almost certain that they are responsible for the digestion of the cuticle, and their secretion must therefore contain both a chitinase and a proteinase.[1] (Perhaps each type of gland is responsible for one of these enzymes, but this possibility has not been proved experimentally.)

The properties of the moulting fluid have been further studied by stripping off the old cuticle from the fifth nymph a few days before moulting and collecting the fluid in a capillary tube. It is a neutral fluid (pH about 7 as tested with phenol red and B.D.H. universal indicator; litmus being turned slightly bluish); it appears to be free from chloride, giving no precipitate with silver nitrate and nitric acid, but it shows the protein colour reactions.

Only the endocuticle is digested by the moulting fluid; the epicuticle is quite untouched, a fact which can be very clearly demonstrated in the following way. If the old cuticle is stripped off in the last few days of moulting, a very delicate moist pellicle usually separates from its inner surface. This is the innermost layer in process of digestion. If it is mounted in water and examined microscopically, it is found to bear a great number of little sacs connected to fine ducts (Text-fig. 10 A, *s*). These are the linings of the old dermal glands that have been cast; they belong to the epicuticle and have so escaped digestion.[1] Their epicuticular nature is readily shown by heating this membrane for a minute or so in strong potash and then treating with iodine. The membrane gives a violet colour; the saccules and ducts stain bright yellow. On further heating in potash they dissolve entirely.

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[1] A minute fragment of congo red fibrin prepared by the method of Roaf (1908) was inserted beneath the old cuticle of a fifth nymph three days before moulting. When the skin was cast three days later, there was a small pink stained area on the cast skin at the site of the fibrin particle.

Were the moulting fluid capable of digesting the epicuticle, there is no apparent reason why the new cuticle also should not be digested. It is protected by the epicuticle being laid down first (see p. 286).

Now the quantity of fluid present at a given moment is very small; it amounts to little more than a moist film (see Text-fig. 9 B and D). It is obvious, therefore, that if the relatively massive endocuticle is to be digested and absorbed, there must be a continuous circulation of the moulting fluid carrying the digestion products into the body of the insect; and this raises the question of the route by which this absorption takes place.

The only author who appears to have considered the question of absorption of the moulting fluid is Wachter (1930), who gives a very good description of moulting in the silk-worm; but she was concerned not with a circulation of the fluid but only with the absorption that takes place just before ecdysis, and she found that in the silk-worm much of the fluid is then swallowed. It seemed very unlikely that it should be ingested by the mouth in Rhodnius, and this possibility was readily excluded by tying a tight ligature round the front of the head at an early stage of moulting. The absorption of the old cuticle and the casting of the skin occurred normally, although the front part of the head was missing and the mouth occluded.[2]

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[1] That the ducts of the moulting glands are not dissolved by the moulting fluid was observed also by Plotnikow (1904).

[2] The insect did not extricate itself completely, and the wings were not fully expanded; but this experiment shows incidentally that in

The question was then studied by taking a number of fifth nymphs at the twenty-fifth day after feeding (that is about three days before moulting), making a small cut with scissors along the margin of the abdomen without injury to the new cuticle, and injecting beneath the old cuticle a saturated solution of neutral red in distilled water. This stained the old endocuticle around the site of injection deep red. Insects dissected next day showed neutral red in the lower segments of the Malpighian tubes (Wigglesworth, 1931) and a diffuse staining of the new cuticle with the dye round the site of injection.

On succeeding days, as the old cuticle was digested and absorbed, the colour at the site of injection became less, and when insects moulted the old cuticle showed only a very faint colour and there was a slight diffuse staining of the new cuticle.

The same results were obtained with indigo-carmine, which appeared in crystalline form in the Malpighian tubes although this dye seemed to inhibit to some extent the digestion of the cuticle around the site of injection.

These experiments demonstrate clearly the continuous absorption of the fluid, and they suggest very strongly that the absorption takes place through the general surface of the new cuticle.

If this is so, the new cuticle cannot attain its waterproof properties until the very end of the moulting period, and that this is the case can be shown by stripping the old cuticle away from the abdomen a day or two before moulting. Within twenty-four hours the abdomen is greatly shrunken and desiccated, although the insect remains alive for several days. It is true that at this stage the endocuticle is not fully formed, but it is almost certain that in the normal insect the impermeability of the cuticle to water is due entirely to the epicuticle or 'Grenzlamelle' (Kühnelt, 1928).

It is thus pretty certain that the function of the dermal glands in Rhodnius is to digest the old cuticle and make it available to the insect for the formation of the new cuticle; a process

Rhodnius the swallowing of air is not an essential process, at least in the early stages of casting the old skin.

which forms an interesting commentary on the view that chitin in insects is a waste product (Bounoure, 1919) and ecdysis an excretory phenomenon.

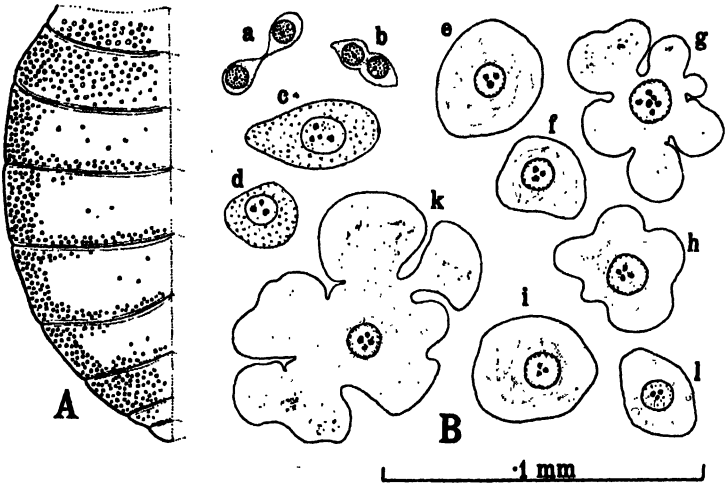
But one of the objects of the present investigation was to find out whether the function of the dermal glands in Rhodnius was the same as that recently demonstrated by Yonge (1932) in Decapod Crustacea, namely, to form the outermost layer of the cuticle (epicuticle) by pouring their secretion over the surface of the endocuticle; or perhaps to contribute to the epicuticle those constituents which render it waterproof. It will therefore be desirable to give the reasons for abandoning this hypothesis.

We have seen that the secretion in the ducts of the dermal glands is strongly basophil; it stains just like the moulting fluid and the innermost layer of the old cuticle as it is being dissolved (Text-fig. 9, *d*), whereas the new epicuticle is unstained during the later part of moulting when the dermal glands are most active. Furthermore, there is no reason to doubt that the basophil secretion which is present around the orifices of the ducts in the adult, and which collects within an hour or two after moulting, represents the normal product of the glands; and this secretion remains soft and basophil throughout the life of the insect. It never hardens into a waterproof layer like the epicuticle.

7. THE OENOCYTES.

The oenocytes in Rhodnius are all confined between the basement membrane and the epidermis; so that by stripping off the tergites and sternites of the abdomen and mounting them whole, it is possible to expose the entire oenocyte system in a single preparation. Their distribution is the same at all stages of the life-history. On the tergites they are concentrated at the sides, along the intersegmental membranes, and all over the first segment (Text-fig. 11 A). On the sternites their distribution is similar, but the concentration in the first segment is not so marked.

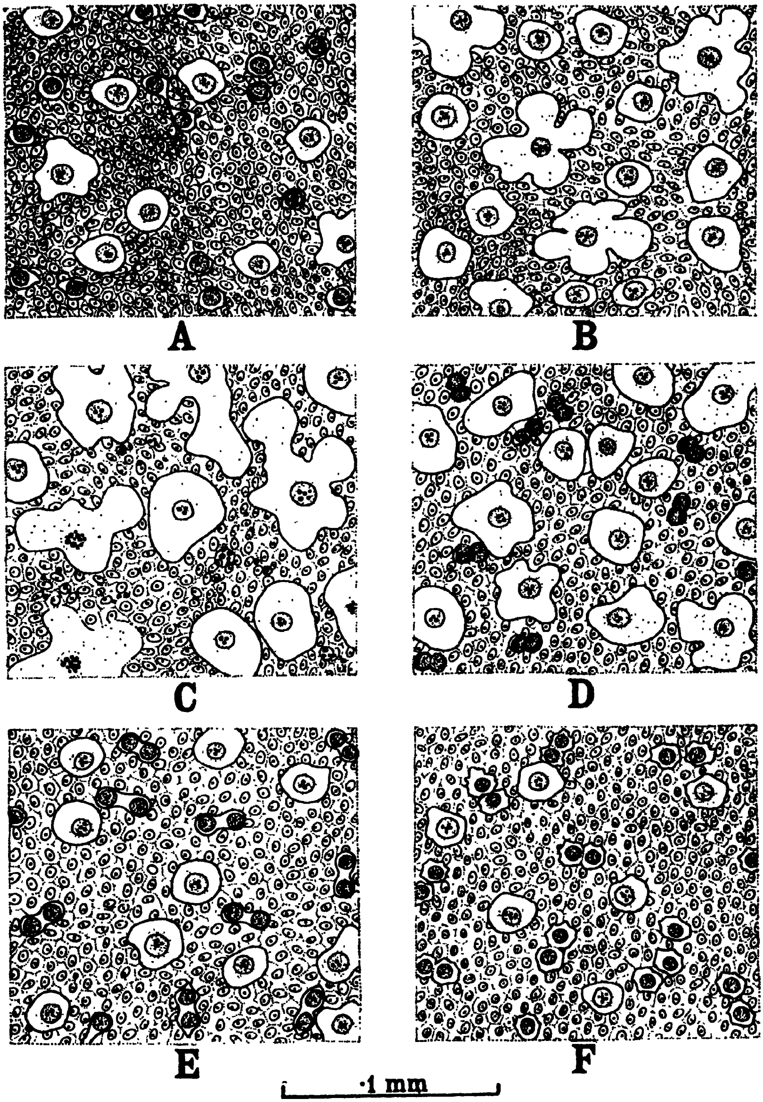
They are very variable in size and shape, sometimes reaching a diameter of 100 μ. Their eosinophil cytoplasm is usually homogenous, or very finely vacuolated; occasionally it is faintly granular (Text-fig. 11, *c* and *d*); often, in fixed material, it shows spindle-shaped clefts or canals (which may be artefacts), as described in Thrixion by Pantel (1898) (Text-fig. 11, *e*, *f*), and sometimes it contains elongated, apparently crystalline bodies, best seen in fresh preparations, like those described in many insects by Hollande (1914) (Text-fig. 11, *h*, *i*). The cytoplasm is not naturally yellow as it is in many insects, nor does it contain granules of pigment.



TEXT-FIG. 11.

A. Distribution of oenocytes on abdominal tergites of fifth nymph (schematic). B. Forms of oenocytes as seen in fifth nymph. *a–d*, at time of feeding; *e–g*, six days after feeding; *h*, fourteen days; *i–k*, twenty-six days after feeding.

Changes in the Oenocytes during Moulting.—During moulting the oenocytes go through a well-defined cycle, which is illustrated in the case of the fourth nymph in Text-fig. 12. At the time of feeding (Text-fig. 12 A) they are of two kinds: (*a*) large solitary more or less lobulated forms with the chromatin massed chiefly near the centre of the nucleus, and (*b*) small rounded forms, evidently a new generation, which are nearly always in pairs and are sometimes united by an unbroken strand of cytoplasm; in these the chromatin granules are more evenly distributed over the nuclear membrane.



TEXT-FIG. 12.

Changes in oenocytes during moulting of fourth nymph. A. Before feeding. B. Five days after feeding. C. Nine days after feeding; several cells showing chromatolysis; chromatin droplets free among epidermal cells. D. Twelve days after feeding; new generation present in pairs. E. At time of moulting. F. Six days after moulting.

After feeding, the oenocytes grow rapidly and the nuclei of the small forms acquire the characters of the large forms (Text-fig. 12 B). By the ninth day (Text-fig. 12 C) they have attained their maximum size, and most have lobes like pseudopodia projecting from their periphery. At this stage many of the older generation break down completely; the cytoplasm disintegrates, and the chromatin of the nuclei collects in deeply staining spheres; the nuclear membrane then dissolves, and these spheres are set free among the bases of the epidermal cells. It is difficult to judge how many of the oenocytes disintegrate in this way, but I am of the opinion that none of the young generation are concerned and only a part of the older generation.

(Chromatolysis and dissolution of a certain number of the oenocytes with the formation of 'chromatin droplets' was described by Weissenberg (1907) during pupation of the Chalcid Torymus and by Stendell (1912) in Ephestia. Poyarkoff (1910), Pérez (1910), Hufnagel (1918), Poisson (1924), and others have observed spheres or droplets of this type among the epidermal cells of various insects at the time of moulting, and have usually regarded them as waste products discharged from the nuclei during a process of rejuvenation. We shall return to the matter of these spheres in dealing with the basement membrane and its cells.)

After the 9th day the oenocytes gradually become smaller. Many large lobulated forms persist until the twelfth day or later (Text-fig. 12 D), but by the time of moulting, at the fifteenth day (Text-fig. 12 E) they are markedly reduced. Reduction continues for a few days after moulting (Text-fig. 12 F), and then they show no further change until another meal initiates a new moulting cycle.

It can now be seen (Text-fig. 12 F) that a new generation of oenocytes has arisen; young forms lie in pairs between the older forms, just as these lay among their predecessors before the last moult began. If the series of preparations is traced backwards in the reverse order, it is found that these young oenocytes have not arisen from the older generation of oenocytes, but from undifferentiated cells in the epidermis. These paired cells can be recognized as early as the tenth day, but before this they cannot be distinguished with certainty from the cells which are going to form the new dermal glands.

Thus a new generation of oenocytes arises from the epidermis at each moult, although some of the old oenocytes also persist. This recalls the conditions described by Weissenberg (1907) in the Chalcid Torymus at the time of pupation, when the imaginal oenocytes arise from the epidermis, and a part only of the larval oenocytes disintegrates. It appears to be a general rule in metabolic insects (see Karawaiew (1898) in Lasius (Hym.), Verson (1900) in Bombyx mori, Kreuscher (1922) in Dytiscus, &c.) for a second generation of oenocytes to arise from the ectoderm at the time of pupation; but the appearance of a new generation at each moult, such as occurs in Rhodnius, does not seem to have been described before, although Poisson (1924) notes that, in Notonecta, oenocytes continue to arise from very small sub-epidermal cells throughout post-embryonic life.

During the moulting of the fifth nymph the oenocytes go through the same cycle. The young cells grow rapidly and reach a maximum at about the fourteenth day, when many of the old cells break down; they remain very conspicuous until about the twenty-fourth day, and during the last few days they become reduced in size. During the moulting of the fifth nymph, however, no new forms appear, so that the oenocytes present in the adult are mainly those which arose during the moulting of the fourth nymph. This is analogous to what happens in metabolic insects, where the so-called 'imaginal oenocytes' always arise at the time of pupation, that is, during the penultimate moult.

Cyclical changes of a more or less similar nature and associated with the moulting rhythm were described by Verson and Bisson (1891) in the oenocytes of the silk-worm, and more recently by Albro (1930) in the Chrysomelid beetle Galerucella. No other authors seem to have found any strict relation between the secretory changes in the oenocytes and the moulting cycle, although changes in volume during metamorphosis have been very commonly observed.

Behaviour of the Oenocytes in the Adult.—The oenocytes persist throughout the life of the adult Rhodnius; but this does not necessarily imply that they are still functional at this stage, for the epidermal cells and the matrix cells of the tracheae also persist, although their function of laying down the cuticle is completed a few days after the final moult. The most important question is whether the oenocytes show signs of renewed activity at any stage, and to decide this they have been examined in both sexes at all periods of fasting, digestion, and reproduction.

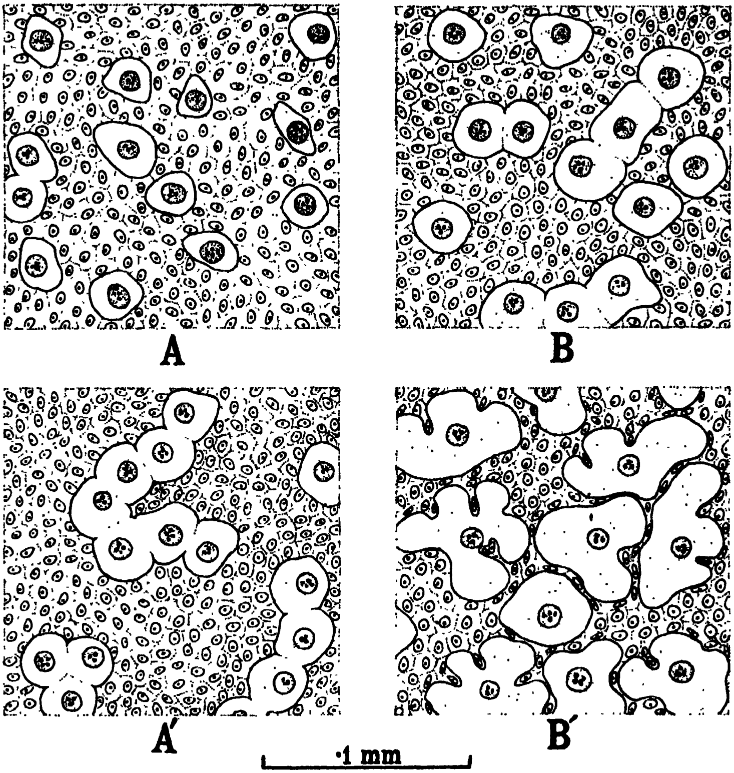
After the final moult, the oenocytes in both sexes tend to lose their regular distribution and collect together into clumps, in which the cell boundaries are very difficult to detect. Their cytoplasm is considerably reduced as compared with their state during moulting, but this reduction is not so extreme in the female. Text-fig 13 A and B shows a typical group of oenocytes in both sexes three weeks after moulting.

At this stage the fasting adults were fed and allowed to mate, and the oenocytes examined on succeeding days. In the males they show only a very slight increase in size, whereas in the females they swell up markedly and develop large 'pseudopodia'. Text-fig. 13 A′ and B′ shows this difference between the sexes ten days after the feed; the same difference persists until the digestion of the blood is complete, that is throughout the period of oviposition.

Function of the Oenocytes.—At the present day there is general agreement that the oenocytes are secretory organs which discharge their products into the blood (Koller, 1929), and their behaviour in Rhodnius during moulting and oviposition leaves no doubt that this view is correct.

The chief question is the nature or function of their secretion. Koller (1929) has suggested that it may be a hormone which induces moulting; but in the case of Rhodnius, moulting, as indicated by the mitoses of the epidermal cells, has begun within five or six days after feeding, whereas the secretory activity of the oenocytes does not reach its maximum until about the ninth day in the fourth nymph and the fourteenth day in the fifth nymph. Moreover, the bulk of the oenocytes in relation to the size of the insect is so great that it seems more likely that they are contributing some material substance to the insect body, than that they are producing only a physiological stimulant or hormone.

Now the cycle of secretory activity in the oenocytes of



TEXT-FIG. 13.

A. Oenocytes in adult male, three weeks after moulting, unfed. B. Ditto, in female. A′. Oenocytes in adult male, ten days after feeding (thirty-one days after moulting). B′. Ditto, in female.

Rhodnius is clearly associated with moulting, and not merely with digestion and assimilation. For in the first place, the oenocytes do not reach their maximum until ten days or so after the meal; and in the second place, when the insect moults, the stomach [1] still contains a large amount of undigested blood which is assimilated during the ensuing weeks. Yet the oenocytes decline markedly as soon as moulting has occurred; indeed, they are becoming reduced before the old skin is cast. They cannot, therefore, be concerned in any of the ordinary processes of intermediary metabolism; they must contribute something specific to ecdysis.

Now the most important changes in the moulting of a non-metabolic insect like Rhodnius are the growth of the epidermis, the digestion of the old cuticle, and the formation of the new. We have seen that the oenocytes show their greatest activity after the growth of the epidermis is complete; they cannot, therefore, be concerned in the process of growth as such. We have seen that the dermal glands are probably responsible for the digestion of the old cuticle, and that there is certainly no 'genetic relation' between the oenocytes and the dermal glands, such as Kremer (1925) claims to have shown in Melasoma (Col.). Moreover, if the oenocytes were connected with the digestion of the cuticle, it would be difficult to understand their renewed activity in the adult female. It is therefore reasonable to suppose that the oenocytes are concerned in the formation of the new cuticle; and that they synthesize, and secrete into the blood, materials which go to form a part of this cuticle.[2]

There are certain facts which favour this hypothesis. (i) The oenocytes are specialized epidermal cells, and this suggests that they have some functional relation with the cuticle. (ii) The oenocytes reach their maximum size just before the new cuticle is laid down (at the ninth day after feeding in the fourth nymph, and the fourteenth day in the fifth nymph). (iii) They become reduced as the cuticle is formed; and this reduction continues, as does the formation of the cuticle, for some days after moulting.

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[1] The mid-gut of Rhodnius consists of a capacious 'stomach' and a narrow convoluted 'intestine'. The blood is stored in the stomach but is not digested until passed on into the intestine. It may take five or six weeks before this store of undigested blood in the stomach is exhausted.

[2] A similar suggestion was put forward in the posthumous paper by Willers (1916).

(Stendell (1912) showed that in Ephestia (Lep.), the oenocytes are large and active during intra-oval development, when the cuticle of the larva is being formed, but they are greatly reduced in the newly hatched larva.)

The next question is the behaviour of the oenocytes in the adult. In both sexes, during reproduction, there is abundant formation of new tissue (eggs and spermatozoa), and in both there is digestion, excretion, and the synthesis of fat &c., in the fat body; but the oenocytes become conspicuously active again only in the female. Now the most obvious difference between the sexes, from a chemical point of view, is the formation of the egg-shells by the follicular cells of the female, and the egg-shells have much in common with the cuticle of the insect itself. They do not contain chitin, but they are composed of an insoluble protein and are covered by a very delicate outer membrane[1] which corresponds with the 'cuticulin' of the insect's epicuticle.

If, therefore, it is supposed that the oenocytes synthesize the protein or the 'cuticulin' elements of the cuticle, then both the cycle of their activity during moulting and their renewed activity in the adult female are understandable. It is reasonable to suppose that by relieving the epidermal cells themselves of this synthetic function, the oenocytes will accelerate the actual deposition of the cuticle. The extraordinary rapidity with which the solid substance of the cuticle is laid down may be judged from Text-figs. 7 and 9.

Whether this hypothesis will obtain support from other groups of insects only further observations can show. There is little information available on the behaviour of the oenocytes in the adult insect, and still less on sexual differences. Wielowiejski (1886) describes differences between the oenocytes of male and female Tipula, but does not clearly state that they are larger in the female. Hollande (1914) records that in many insects the oenocytes diminish in volume after mating in the male and oviposition in the female. Kremer (1925) observed that in young adults of Melasoma (Col., Chrysomelidae), in the autumn, the oenocytes show a progressive reduction in size in spite of the insect taking in a large supply of food, and the fat body becoming enlarged ready for hibernation. But during sexual activity in the following summer they become enlarged again, and this enlargement is greater in the males. The conditions here, however, are not quite comparable with those in Rhodnius because Kremer describes as oenocytes the enlarged fat body cells charged with albuminoid materials. He regards the oenocytes of the adult beetle as metabolic intermediaries ('Vermittler') between the fat body and sexual organs; at the time of mating they are said to have practically disappeared.

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[1] If the empty shell is immersed in strong nitric acid, it becomes bright yellow and bubbles of gas are evolved. But these bubbles come only from the inside of the egg, and when they have ceased to come off there remains this delicate outer membrane. It is insoluble in strong hydrochloric and sulphuric acids in the cold, dissolves readily in alkali, the biuret reaction is negative, and it gives a yellow colour with iodine.

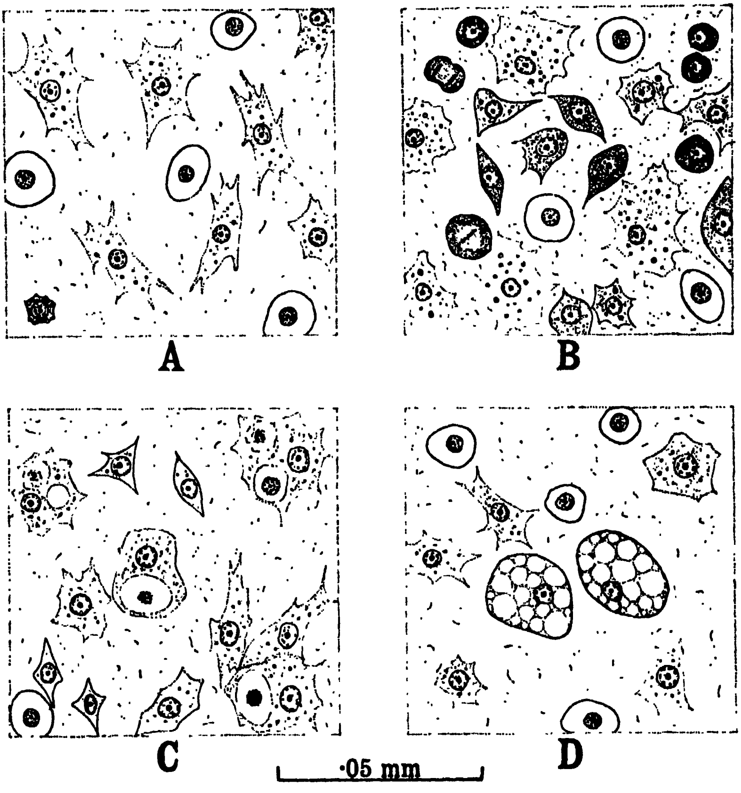
8. THE BASEMENT MEMBRANE AND HAEMATOCYTES.

Text-fig. 14 A shows the appearance of the basement membrane in the fourth nymph at the time of feeding. Two chief kinds of cell occur on its surface: stellate cells with radiating processes and granular inclusions, and rounded cells with clear eosinophil cytoplasm devoid of granules. Here and there, especially around the tracheae where they pierce the basement membrane and along the sides of the dorsal vessel, there are clumps of haematocytes of varied form and size (Text-fig. 15 A).

A few days after feeding, all these cells begin to multiply exceedingly and numerous mitotic figures are seen; by the fifth day many parts of the basement membrane are covered with densely packed cells, which present a most varied appearance. Many of them are of the stellate type described above; most are spindle-shaped or pyriform, and scattered through them are always some of the rounded eosinophil type.

The time of greatest development of these cells is reached about the eighth or ninth day (Text-fig. 14 B), when the oenocytes are breaking down and the 'chromatin droplets' are most abundant in the epidermis. At this time many of the cells below the basement membrane become charged with similar blue staining droplets or granules.

After the tenth day, when the 'chromatin droplets' are no longer present in the epidermis, many of the cells on the basement membrane begin to degenerate and they are then ingested by their neighbours (Text-fig. 14 c). In this way their numbers become greatly reduced, and by the time of moulting they are

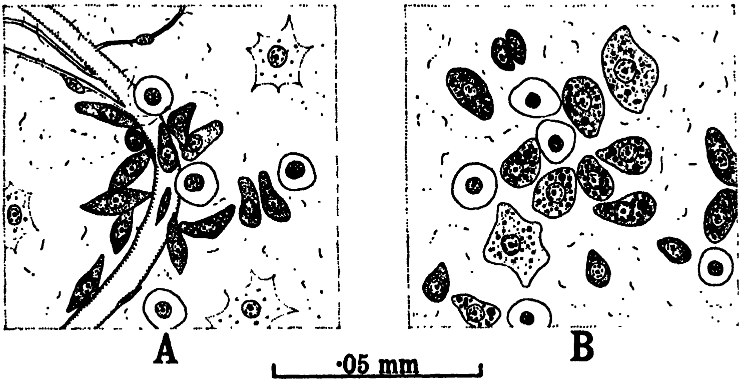


TEXT-FIG. 14.

Changes in haematocytes on basement membrane of fourth nymph during moulting. A. At time of feeding. B. Nine days after feeding; showing mitosis of 'proleucocytes', 'phagocytes' of many forms containing basophil droplets, and 'oenocytoids'. C. Twelve days after feeding; many of the 'phagocytes' contain the degenerating bodies of other cells. D. Twenty-four hours after moulting, stellate 'phagocytes', 'oenocytoids', and 'adipoleucocytes' present.

relatively sparse (although occasional mitoses can still be seen), save in the occasional clumps around the tracheae and at the sides of the dorsal vessel.

If Indian ink is injected into a nymph at, say, twenty-four hours after feeding, most of the stellate cells forsake the basement membrane, take up particles of the Indian ink and collect in clumps, like the haematocytes, along the dorsal heart and elsewhere.[1] But the round eosinophil cells do not engage in this phagocytic activity (Text-fig. 15 B).



TEXT-FIG. 15.

A. Trachea passing through basement membrane in fifth nymph, at time of feeding. Stellate phagocytes and oenocytoids on surface of the membrane; a clump of fusiform and pyriform phagocytes around the trachea. B. Clump of cells on basement membrane of fifth nymph twenty-four hours after injection of Indian ink (forty-eight hours after feeding). All cells contain Indian ink except the small proleucocytes and the oenocytoids.

These observations suggest that all these cells are simply haematocytes, which multiply and collect over the surface of the basement membrane during moulting because that is where their phagocytic activities are in greatest demand.

On the other hand, the appearance shown in Text-figs. 14 A and B recalls that described by Mayer (1896), and suggests that the stellate cells at least are matrix cells of the basement membrane, which might be supposed to arise from their modified cytoplasm.

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[1] Muttkowski (1924) has shown how amoebocytes in insect blood apply themselves to smooth surfaces and assume the stellate form described above.

If the views of Lazarenko (1925) are correct, there is nothing incompatible about these two alternatives. For, according to this author, the connective tissue membranes of insects (of which the epidermal basement membrane is a typical example) are formed in just this way—by the transformation of the cytoplasm of phagocytic blood-cells.

All the forms present among these cells can be readily homologized with the leucocytes described by Hollande (1911) in Pyrrhochoris apterus. (i) The small forms with little cytoplasm and large nucleus, frequently seen dividing, are the 'proleucocytes'; (ii) the larger forms showing endless variation in shape and size are the 'phagocytes'; and (iii) the round forms with eosinophil cytoplasm are the 'oenocytoids'. Finally (iv) there appear, especially towards the end of the moulting period (Text-fig. 14 D), large forms with abundant fat droplets in the cytoplasm. These are the 'adipoleucocytes' of Hollande, the ultimate fate of which in Rhodnius has not been traced.

The function of the rounded 'oenocytoids' is unknown; they certainly have no connexion with the oenocytes as suggested by Poisson (1924) for similar cells in the aquatic Hemiptera. But all the other types appear to take up and digest the cellular débris resulting from the breakdown of the oenocytes and the old dermal glands and possibly some of the epidermal cells themselves. Poyarkoff (1910), Pérez (1910), and Hufnagel (1918) have described how the basophil spheres and granules in the epidermis of Galerucella (Col.), Calliphora (Dipt.), and Hyponomeuta (Lep.) are taken up by the phagocytes; and the role of phagocytes in the histolysis of the muscles of Diptera and Hymenoptera is well known (Pérez, 1910, 1911). The only difficulty about this view is that in Rhodnius the basement membrane appears always to be a continuous unbroken sheet.

During moulting in the fifth nymph, the haematocytes go through the same cycle of changes. They multiply to reach a maximum about the fourteenth day, becoming filled with granular débris at this time, when the oenocytes, &c., are breaking down; they then become reduced by ingesting one another.

The same wide range of forms is present as in the fourth nymph. In the adult they forsake the basement membrane, for the most part, and persist chiefly in clumps along the tracheae and at the sides of the dorsal heart. They do not show any marked increase after feeding or during oviposition. Their activity is clearly associated with moulting and not merely with digestion and assimilation.

9. Summary and Conclusions.

1. Structure and Composition of the Cuticle.

The cuticle of Rhodnius consists of two primary layers: a very thin epicuticle ('Grenzlamelle') and a relatively thick endocuticle traversed by fine pore canals containing protoplasmic filaments or fluid.

The endocuticle is composed of protein and chitin. The epicuticle is composed of material for which the name 'cuticulin' is proposed, which has chemical properties like those of the cutin or suberin of plants and is perhaps a complex mixture of fatty or waxy substances. Melanin is often present in the epicuticle.

The endocuticle is made up of two parts separated by a distinct horizontal line; of these, only the outer is laid down before moulting. The endocuticle shows also rather faint horizontal laminae.

In the adult, over the greater part of the abdominal tergites and sternites, the outer part of the endocuticle is impregnated with 'cuticulin' more or less mixed with melanin. This layer corresponds with the 'Emailschicht', 'Pigmentschicht', or 'exocuticle' of various authors. In the nymphs, the endocuticle of the abdomen is impregnated with cuticulin only below the little plaques which bear the bristles. Consequently, the stretching of the abdomen to receive the large meals of blood is accomplished by a different mechanism in nymphs and adult (p. 276).

The cuticle is traversed by numerous ducts opening to the exterior. In the nymphs and in the abdominal sternites of the adult, these are evenly distributed; in the tergites of the adult they occur chiefly at the sides of the abdomen. Their distribution is not related to the pigmentation of the cuticle.

Around the orifice of each duct is a little patch of dried secretion which stains readily with methylene blue. This corresponds, presumably, with the 'Sekretschicht'; but in Rhodnius it is not an integral part of the cuticle.

2. Structure of the Epidermis (hypodermis).

This consists mainly of conical cells containing granules of a brick-red pigment; but beneath the bristles, and in some other places, the cells are stuffed with spheres of uric acid. Among the epidermal cells are the dermal glands, the oenocytes, and embryonic cells which give rise to new dermal glands and oenocytes at the time of moulting. The basement membrane is well developed, and many haematocytes adhere to its lower surface.

3. The General Process of Moulting.

After a full meal, the fourth nymph moults, as a rule, in fifteen days at 24° C. and the fifth nymph in twenty-eight days. These times do not seem to be affected by the duration of the fast preceding the meal.

4. The Epidermis, Red Pigment, and Uric Acid Cells.

The epidermal cells swell up within a day or two after feeding; then they separate from the cuticle, and multiply by mitosis from the fifth to the ninth day in the fourth nymph and from the sixth to the fifteenth day in the fifth nymph. The red pigment and the uric acid both increase until about the eighth day in the fourth nymph and the twelfth day in the fifth nymph. The uric acid then decreases rapidly; the red pigment more slowly.

It is suggested that both the uric acid and the red pigment may be waste products of the synthesis of various constituents of the cuticle. They reach a maximum shortly before the cuticle is laid down.

5. The Formation of the New Cuticle.

The non-chitinous epicuticle is laid down first. It appears as a smooth membrane which later becomes folded, particularly in the nymphs. The chitinous endocuticle is laid down below the epicuticle. It appears to be secreted in fluid form around filamentous processes from the epidermal cells which become the pore canals.

The active intervention of the underlying cells, effected through the endocuticle, presumably by way of the pore canals, is necessary for the pigmentation of the epicuticle at the time of moulting. The impregnation of the outer part of the endocuticle with 'cuticulin' also appears to take place gradually after the endocuticle is formed. It is suggested that the function of the pore canals may be to permit this action at a distance.

The bristles are formed by trichogen cells; these give out processes which pass through annular 'tormogen' or socket-forming cells and later become chitinized and impregnated with cuticulin.

6. The Dermal Glands.

These are associated with moulting, and afterwards degenerate. They are formed anew at each moult from embryonic cells in the epidermis. Their development and structure is described. They are of two distinct types, one of which is much more numerous than the other.

These glands secrete the moulting fluid, the chief function of which is to digest the old cuticle. Their secretion must contain a proteinase and a chitinase. All but about 14 per cent. of the old cuticle of the abdomen is digested and absorbed; but the epicuticular structures, such as the linings of the ducts of the old dermal glands, are not affected. Experimental evidence is given that there is a continuous circulation of the moulting fluid, and that the products of digestion are absorbed through the general surface of the new cuticle, which is shown not to develop its waterproof properties until the time of moulting. When the skin is cast, practically no moulting fluid is present.

The dermal glands appear to play no part in the formation of the new cuticle.

7. The Oenocytes.

The oenocytes are all confined between the basement membrane and the epidermis. A new generation arises at each moult (except the last) from the embryonic cells in the epidermis.

During moulting they go through a definite cycle, growing rapidly and reaching a maximum just before the new cuticle is laid down; that is, at the ninth day in the fourth nymph and the fourteenth day in the fifth nymph. At this stage many of them undergo chromatolysis with the liberation of chromatin droplets. From this point until the time of moulting the survivors gradually become reduced in size.

The oenocytes persist in the adult; after feeding, they become conspicuously active again in the female, but show very little change in the male.

It is suggested that they synthesize some of the (non-chitinous) constituents of the cuticle during moulting, and of the egg-shells during maturation of the ova.

8. The Basement Membrane and Haematocytes.

The cells of the basement membrane appear to be haematocytes. The forms of those present are described.

During moulting they increase greatly by mitosis, and the phagocytic forms become charged with blue staining granules and débris possibly derived from the chromatolysis of the oenocytes, old dermal glands, and perhaps some of the epidermal cells themselves. In the later stages of moulting their numbers are reduced. Many of them degenerate and are ingested by the survivors.

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Notes on Odontosyllis.

By

Edwin S. Goodrich, F.R.S.

With 12 Text-figures.

During a recent visit to the Biological Station in Bermuda, my attention was drawn to the interesting 'fire-worm', Odontosyllis enopla Verrill, by Dr. E. L. Mark, who kindly helped me to obtain living specimens. The strange swarming habits of this Syllid have been described by Galloway and Welch (1916), who have also given a good general account of its anatomy. Certain points which escaped them, more especially concerning the structure of the alimentary canal and nephridia, seem to be worthy of special notice and are here described.

My material of Odontosyllis enopla consists of mature swarming individuals captured near the surface on July 18 and 19, 1932, some were studied alive, others fixed in Bouin's fluid.

The nephromixia.

Galloway and Welch give a short account of the 'nephridia' (really nephromixia) in the adult, and, while stating that in the males they are distended and serve as sperm-ducts, consider that in the female 'the tubular part was so compressed and displaced as to preclude the possibility that it could be used for the discharge of eggs, at least at the beginning of ovulation'. There is, however, no other exit for the eggs, and an examination of spent and half-spent females shows that in this sex also the nephridial tube is enlarged and serves as oviduct, as it does in other Syllids. Both males and females survive the extrusion of genital products, and probably live to breed again.

In my third contribution 'On the Nephridia of the Polychaeta' (1900), it was shown that the nephridium in immature Syllids is a slender tube with an open nephridiostome having a long projecting ventral lip bearing a 'flame' or bunch of long cilia passing down the lumen, and a short dorsal lip without cilia. Such a nephridiostome is characteristic of the family Syllidae, and resembles that of the smaller Oligochaeta. It was also shown that in mature Syllids, and in the forms which undergo fission into a sexual stock and reproductive stolon, the nephridia become converted into nephromixia by the addition to the original nephridiostome of a relatively large genital funnel, the coelomostome. This represents the funnel of the coelomoduct derived from the coelomic epithelium on the anterior face of the septum to which the nephridium is attached, and through which it projects into the next segment in front. Further, the nephridial canal is enlarged, its wall thickened and often coiled.

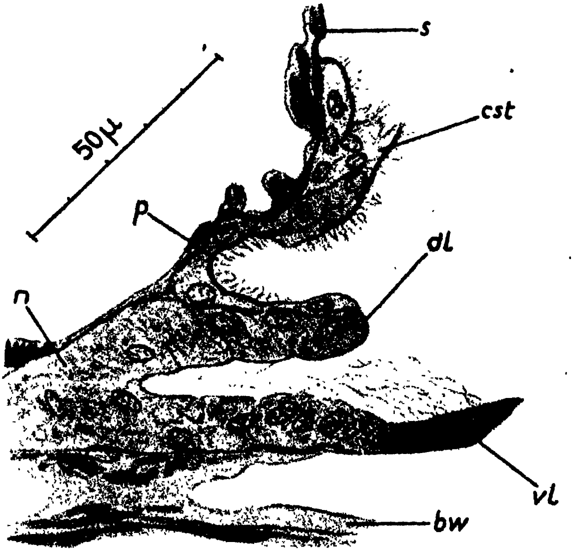
The nephromixium so formed in both sexes carries the genital products to the exterior. Such nephromixia are fully developed only in those segments in which these products are stored, and the nephridia in the quite anterior and posterior segments remain unmodified. Between the fertile and sterile segments intermediate regions of a few segments occur in which the coelomostomes become more and more developed until they reach full size towards the typical genital segments. These observations were confirmed by Fage some years later (1906).

Now, the excretory organs of Odontosyllis enopla conform in general structure to the type found in other Syllids, but present two features of special interest.

Whereas in Syllids generally the original nephridiostome becomes, so far as hitherto observed, indistinguishably fused with the coelomostome when the latter is fully developed, in Odontosyllis enopla it retains its individuality. In the spent female, even in the living state, the ventral lip with its bunch of cilia can be distinguished from the coelomostome itself which bears a dense coating of shorter stiffer cilia of different appearance and action.

The persistence of the nephridiostome is particularly obvious in the case of the female, for here the large coelomostome surrounds the anterior preseptal portion of the nephridium in such a way that a deep pocket is formed dorsally at the bottom of the genital funnel into which projects the nephridial tube (Text-figs. 1 and 2, *p*). Thus, while the ventral lip of the nephridiostome is embraced on both sides by cells of the coelomostome

(Text-fig. 5), the short dorsal lip is situated towards the lumen of the coelomostome funnel. This region of the nephridial canal is covered by the reflected coelomic epithelium (here not ciliated) which reaches the free edge of the dorsal lip. This somewhat complicated relationship of the two funnels is shown



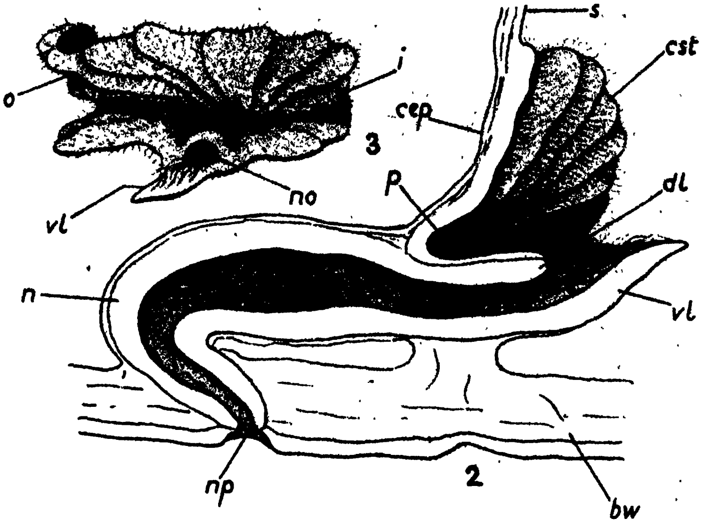
TEXT-FIG. 1.

Longitudinal section through the combined nephridiostome and coelomostome of a nephromixium from the middle genital region of a swarming female Odontosyllis enopla.

LETTERING OF TEXT-FIGS. 1–12.

bw, body-wall; *c*, coelom; *cep*, coelomic epithelium; *cst*, coelomostome; *dic*, dorsal intestinal canal; *dl*, dorsal lip of nephridiostome; *ep*, epidermis; *i*, inner or median edge of coelomostome; *int*, intestinal epithelium; *lbv*, longitudinal dorsal blood-vessel; *lm*, longitudinal muscle; *m*, dorsal mesentery; *mi*, muscular coat of intestine; *n*, nephridial canal; *nc*, lumen of nephridial canal; *no*, opening of nephridiostome; *np*, nephridiopore; *o*, outer or lateral edge of coelomostome; *om*, oblique muscle; *op*, opening of dorsal canal into intestine; *p*, pocket at bottom of coelomostome; *s*, transverse septum; *vl*, ventral lip of nephridiostome.

in longitudinal and transverse sections (Text-figs. 1, 5, 6). Text-fig. 1 is of a sagittal section cutting the combined nephridiostome and coelomostome longitudinally, and showing the persistent ventral lip of the former and the pocket formed dorsally by the latter. The course of the preseptal nephridial canal and position of the nephridiostome can also be followed in the transverse sections drawn in Text-figs. 4 and 5, and in the reconstructions drawn in Text-figs. 2 and 3.

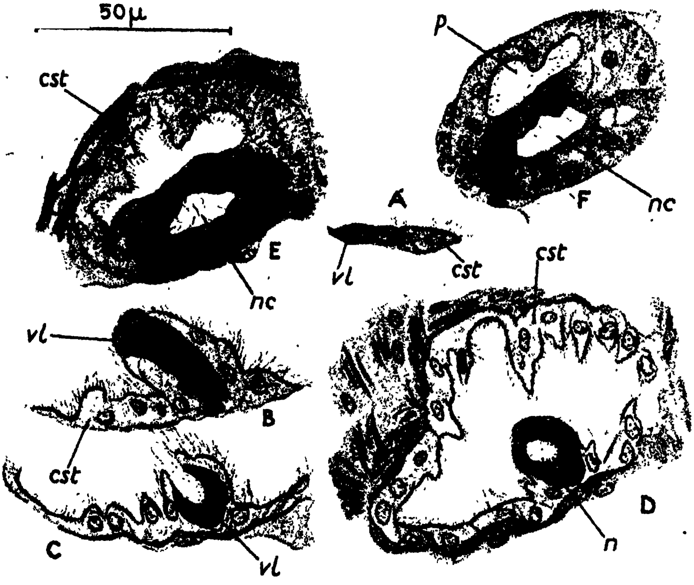


TEXT-FIGS. 2 and 3.

Diagrammatic reconstruction of the nephromixium in longitudinal section. From a genital segment of a female Odontosyllis enopla.

Anterior view of the combined nephridiostome and coelomostome of a nephromixium. Odontosyllis enopla, ♀.

The second point of interest concerns the coelomostome only. It will be remembered that its ciliated surface in many Polychaetes is provided with deep furrows separated by sharp-edged ridges converging towards the bottom of the funnel. Although perhaps of no very great morphological importance, and not of universal occurrence, these ciliated ridges are very characteristically developed in many families widely separated systematically. For instance, they are conspicuous in Nereidae (Goodrich, 1893) and Hesionidae (Goodrich, 1897), and among solenocyte bearing forms the ridges have been described in Nephthyidae (Goodrich, 1897), and recently by A. Meyer (1926) in Tomopteridae. Probably a careful search would reveal their presence in other families.



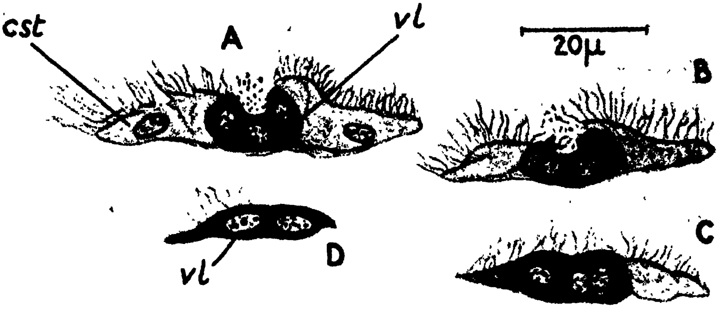
TEXT-FIG. 4.

Transverse sections of the combined nephridiostome and coelomostome. A, most anterior section through ventral lip of nephridiostome and two cells of coelomostome. B, cuts nephridiostome obliquely. C, ventral lip embraced by coelomostome cells. D, beginning of nephridial canal bulging into coelomostome. E and F, formation of blind pocket towards posterior end of coelomostome. Odontosyllis enopla, ♀.

In the mature female Odontosyllis enopla similar sharp ciliated ridges are found on the inner surface of the genital funnel or coelomostome as shown in Text-figs. 1, 2, 3, and 4.

There are about ten main ridges running from the lip towards the bottom of the funnel and opening of the nephridial canal. No doubt they serve to facilitate the passage of the genital products to the exterior. In the male they are much less marked.

In the female the nephridial canal has a simple bend; Text-fig. 6 shows a transverse section of it, and the section in Text-fig. 7 passes through the external pore. The nephridial canal of the male is more enlarged and slightly coiled.



TEXT-FIG. 5.

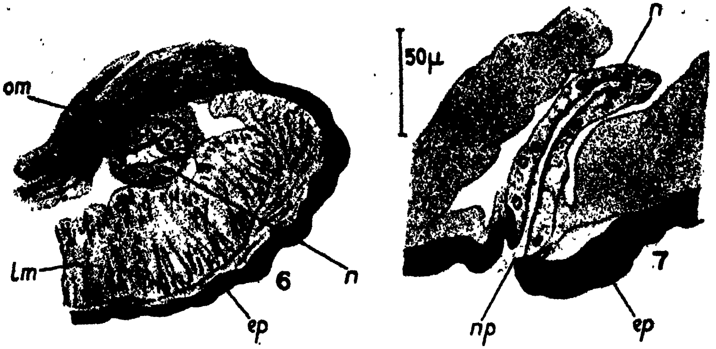
A–D, four transverse sections of lower lip of nephridiostome from behind forwards. Odontosyllis enopla, ♀.

There can be no doubt that the nephromixia of Odontosyllis serve as genital ducts in both sexes, as in other Syllids.

Lastly, it may be mentioned that in my female specimens of Odontosyllis nopla, caught in the act of swarming, all the eggs free in the coelom are, apparently without exception, in the state of arrested first maturation division. They all show a well-formed prophase amphiaster, and presumably the division is not completed and the polar body is not given off until the eggs are shed into the surrounding sea-water.

The dorsal intestinal canal.

When examining transverse sections of Odontosyllis enopla I was astonished to find in the mid-dorsal line a narrow tube running along in the thickness of the intestinal wall. On looking up Galloway and Welch's paper (1916) I found that they described and figured the canal, but, strangely enough, mistook it for the dorsal longitudinal blood-vessel. This vessel of course is present, but runs as usual outside the gut in the dorsal mesentery (Text-figs. 11 and 12, *lbv*). Since no such dorsal canal could be found described in any other Syllid, or indeed in any other Polychaete, I at first thought it was something new to science. But, quite recently, Arwidson (1932) has described a similar tube in a new parasitic Syllid, Calamyzas amphictenicola. It is absent in all the other genera of the Syllidae of which I have sections—such as Syllis, Haplosyllis, and Pionosyllis. On the other hand, a dorsal intestinal canal is present in all the species of the genus Odontosyllis I have been able to examine.[1]



Text-figs. 6 and 7.

Transverse section showing canal of nephromixium. Odontosyllis enopla, ♀.
Transverse section through nephridiopore. Odontosyllis enopla, ♀.

In Odontosyllis enopla the dorsal intestinal canal runs

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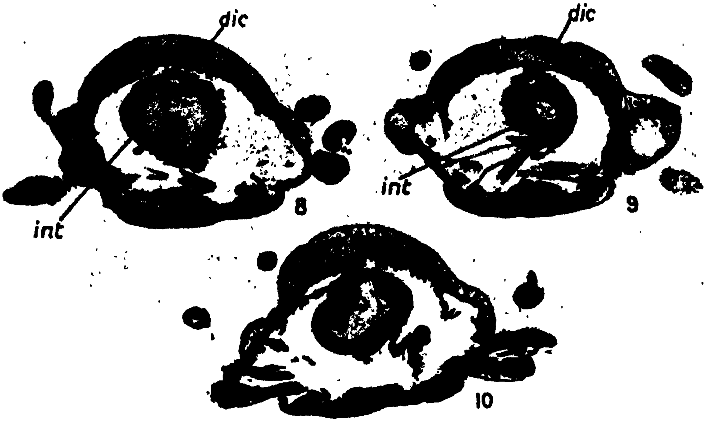
[1] Mr. F. A. Potts kindly showed me his sections of Odontosyllis phosphorea Johnston, from Namaino. To Dr. R. Herpin and Mr. C. C. A. Munro I am indebted for specimens of Odontosyllis ctenostoma Clap. from Cherbourg, and to Dr. E. J. Allen for Odontosyllis fulgurans A. and E. from Plymouth.

along the whole length of the true intestine, from its anterior end to the beginning of the posterior rectal region. Throughout its length its lumen is separate from the main cavity of the gut. The canal lies embedded in the wall of the intestine just internal to the intestinal muscular layer. It is enclosed by its own wall and by the cells of the intestinal epithelium which meet below it (Text-figs. 8, 9, and 11). Although the wall of the canal is composed of cells of the same nature as those of the intestinal epithelium, they are smaller, flatter, less granular, and more sparsely ciliated. Some fourteen to twenty segments from the pygidium the intestine narrows, its wall thickens, its lumen diminishes and becomes circular in section (Text-fig. 9). Posteriorly to this short narrow region the intestine expands again to form the rectal region. It is here that the dorsal canal opens by a wide aperture into the main lumen (Text-figs. 10 and 12).



Text-figs. 8, 9, and 10.

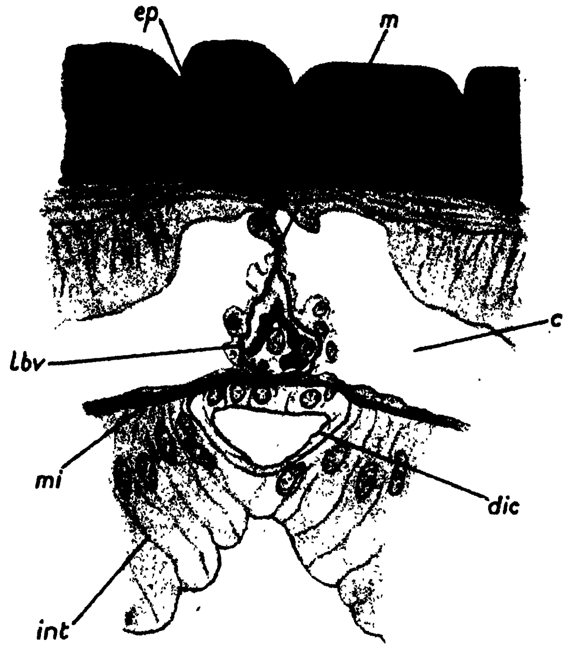
Microphotographs of transverse sections.

Fig. 8. Mid-region of intestine.

Fig. 9.—Narrow thick-walled region of intestine leading to rectal region.

Fig. 10.—Anterior part of rectal region showing communication of dorsal canal with intestinal lumen.

Anteriorly the dorsal canal tends to become smaller and more cylindrical. Near its extreme anterior end the intestine enlarges and a circular valvular fold is pushed into its lumen. Here the dorsal canal becoming very small finally disappears, its wall apparently merging into the general intestinal epithelium. No distinct anterior opening of the canal seems ever to be present.

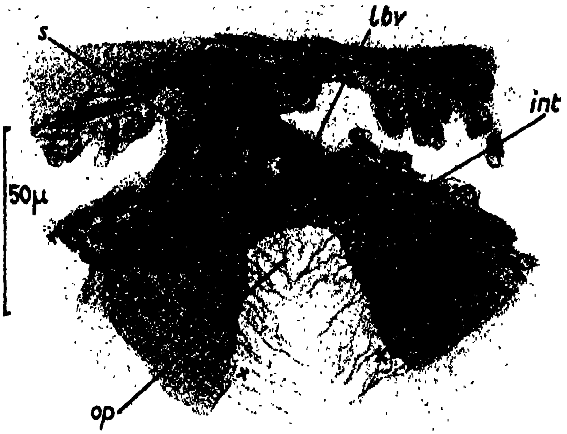


TEXT-FIG. 11.

Portion of transverse section in mid-region, showing dorsal canal in intestinal wall.

At most it appears to communicate with the main lumen by narrow chinks between the cells at its extremity. Short blind lateral sacculations may sometimes be seen near the anterior end of the dorsal canal.

From the appearance of transverse sections it may safely be inferred that the dorsal canal has been formed by the closing of a longitudinal groove of the intestinal epithelium which, however, remains in open communication behind. Until it has been further studied in the living worm it is difficult to suggest what its function may be. Arwidson (1932) failed to find any opening either in front or behind in Calamyzas. In Odontosyllis a fairly wide posterior opening seems to be always present, but the anterior opening if present at all appears to be too small to be of much functional importance unless it can be widened on occasion. The dorsal canal in Odontosyllis can, therefore, not be compared to the ventral 'siphons' of Capitellids and Echiurids. According to my own observations the lumen of the canal is always remarkably free from any solid contents, either in the form of food particles or of precipitates. However, it is difficult to avoid the conclusion that it is in some way concerned with the distribution of nutritive fluid from the intestine, or possibly with the carrying forwards of a respiratory current of sea-water from the rectum.



TEXT-FIG. 12.

Portion of transverse section photographed in Text-fig. 10 showing opening of dorsal canal into intestine. Two x's mark the folds which join forwards to close off the canal.

SUMMARY.

Some new features of interest are described in the excretory organs and intestine of Odontosyllis enopla Verrill, from Bermuda. The nephromixia in the genital region are formed by the combination of the nephridium and the coelomostome in such a way that the original nephridiostome preserves its structure and individuality in the fully developed organ. The coelomostome in the female is large and provided with many ciliated ridges, as in several other families of Polychaeta. The eggs in the ripe swarming female are all in the prophase of the first maturation division.

A longitudinal dorsal canal runs in the thickness of the wall of the intestine, opening behind into the rectal region. It dwindles and disappears at the anterior end of the intestine. Such a dorsal canal is present in all the species of Odontosyllis examined. A similar canal, but without openings, has recently been described by Arwidsson in the Syllid Calamyzas. In Odontosyllis the dorsal canal may carry nutritive fluid forwards, or possibly a respiratory current of sea-water from the rectal region.

University Museum, Oxford.
August 9, 1933.

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Improved Technique for Non-aseptic Tissue Culture of Helix aspersa, with Notes on Molluscan Cytology.

By

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With Plates 19 to 22 and 3 Text-figures.

INTRODUCTION.

In two previous papers the senior author (**3, 4**) and Dr. E. S. Duthie (**5**) have referred to the possibility of obtaining outgrowths of various kinds from pieces of mantle cavity wall of Helix aspersa kept in blood and Ringer's solution.

Hitherto the most successful outgrowths have been obtained with ex-plants mounted in blood, but more recently we have had greater success by using Hédon Fleig Ringer fluid, which is a properly balanced solution with a pH nearly identical with snail's blood. For some reason which we do not at present understand, ex-plants made in Hédon Fleig Ringer are less contaminated by bacteria after some days' growth, and last longer.

The present paper is intended to illustrate the results already obtained, and to give some account of the cytology of the various cell elements encountered in this class of tissue culture. It will be noted from the microphotographs illustrating this paper that we have succeeded in obtaining outgrowths consisting of large masses of cells. Owing to the fact that the mantle cavity is already contaminated by bacteria, all the ex-plants mounted grow in the presence of a bacterial fauna, and none of the usual aseptic precautions has been taken.

All the cultures were made by the junior author, who was also responsible for our observations on the pH. These were kindly gone over by Dr. Joseph Bigger, of the Bacteriology Department. Dr. Emil Werner of the Chemistry Department made some of the rarer chemicals used, and was ever ready with helpful advice, as was Dr. Bigger, and Mr. Wager of the Botany Department.

Some of the osmic acid used for this work was purchased with a grant from the Royal Society Fund, for which we are grateful.

PREVIOUS WORK.[1]

Some of the previous work is reviewed by Craciun (**1**) who may be consulted. The previous work of Fauré-Fremiet on the amoebocytes of Terebella, Arenicola, Asterias, &c., may be mentioned. He distinguishes three types of invertebrates. (*a*) Those with what he calls 'linome' and usually fusiform or membrane-like in appearance. (*b*) Those with no 'linome' but of a similar shape. (*c*) Those with vesicular amoebocytes. The 'linome' of Fauré-Fremiet consists of osmiophile filaments or fibre-like structures in the cytoplasm. Fauré-Fremiet and Dehorne use the word 'choanoleucocyte' for the single type of migratory cell.

In three previous communications the senior author and his pupils have described the appearance of remarkable bladder-like structures from ex-plants of Helix. The reader is referred to a recently published paper on this subject in the 'Journ. Roy. Micr. Soc.' (1932).

The most recent study of molluscan neurones and other cells in which both Golgi bodies and neutral-red granules are dealt with is that by Monné (**10**). This paper, published in a rather inaccessible journal, has been overlooked by Young (**14**). Of the molluscan neurone Monné writes:

Im lebenden Zustande ist der Golgi-Apparat unsichtbar. Das Vacuome lässt sich mittels Neutralrot nachweisen. In kleinen Neuronen bildet das Vacuome eine Ansammlung von Kügelchen, eventuell Vakuolen, welche den Axon abgewendenten Pol der Zelle einnehmen. In grösseren Neuronen ist das Vakuome im ganzen Plasma zerstreut, nur derjenige Plasmabezirk, von welchem das Axon abgeht, wird von Vakuome-Kügelchen gemieden. Der erwähnte Plasmabezirk wird dagegen vorzüglich von lipochromatischen Kügelchen ausgefüllt, welche auch in anderen Plasmateilen, immerhin aber nur in sehr geringer Anzahl, angetroffen werden können. Diese lipochromatischen Kügelchen sind stark lichtbrechend, manchmal orangefarbig. Wir sind mit Parat darin einig, dass sich die Lipochrome mit Neutralrot nicht farben lassen.'

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[1] We have not dealt with Dr. Bohuslav's paper because it has only recently come into our hands. We take this opportunity of thanking Major Paul Ruzicka, Czechoslavakian Consul in Dublin, for getting the paper for us.

He adds, 'Das Golgi-Apparat-Element ist ein typisch flächenhaftes Gebilde. Seine Peripherie wird von einem tiefschwarzen, kreisrunden oder ovalen Ring eingenommen. Dieser Ring umschliesst eine Fläche, welche sich dunkelgrau, hellgrau oder gar nicht mit Osmium färbt.'

Monné describes this type of Golgi apparatus in other varieties of somatic cells of molluscs. In the connective tissue cell he shows that the neutral red may segregate outside the area of the Golgi apparatus.

Young (**14**), to whose paper reference will be made below, seems to have failed to make proper Golgi preparations, and his discussions and statements on the 'Golgi-vacuome' problem lose therefore much of their value. It is interesting to note that Monné gives figures of 'Golgi-Apparat-Elemente mit anliegenden, osmiophilen Kügelchen' occurring in strongly osmicated neurones.

We cannot refer at length to the work of Parat (**11**) which is mentioned below, but we now agree with him that the lipochrome granules do not stain in neutral red. One other study on the Golgi bodies of neurones may be mentioned—that of Kolatchew (**6**). This paper is interesting because it is the first study published with the Champy-Flemming post-osmicated method known by his name. Kolatchew gives figures of snail neurones which we would accept as perfectly accurate. He uses no Golgi method except his own, which, as is well known, often shows mitochondria as well as Golgi bodies in many parts of the tissue. Had Kolatchew used the Weigl-Ludford or Da Fano method he would not have been led to confuse the mitochondria and

Golgi elements which are quite easily separated into two categories by Weigl or formalin silver preparations. In no case are the true mitochondria accompanied by chromophobe substance. Whatever difficulty may be experienced in discriminating between Golgi bodies and mitochondria in Kolatchew slides of neurones, no such trouble will be met with in other tissues.

The most recent work of Saguchi (**12**) on the Golgi apparatus of cells of tissue cultures is interesting. In recent years we have heard that the tissue-culture cell is the only normal cell because it can be examined without manipulation (Walker, **13**). As a matter of fact both Ludford (**9**) and Saguchi (**12**) show that the tissue-culture cell may become very markedly changed from the normal cell that it was. We do not propose to enter into this question in the present paper, but we draw the attention of those who may have been interested in Walker's claims to the recent paper by Saguchi.[1]

Saguchi's work indicates the value of the formalin silver nitrate techniques of Cajal (and Da Fano) when used by a skilful technician, and shows that these methods are adequate to give delicate preparations of such difficult material as tissue cultures.

METHOD.

The photographs given in this paper are all, except fig. 4, Pl. 20, from living cultures made in Hédon Fleig saline, the formula of which for cold-blooded animals used is:

NaCl	7·0 gm.
KCl	0·3 „
CaCl	0·1 „
$NaHCO_3$	1·5 „
Na_2HPO_4	0·5 „
$MgSO_4$	0·3 „
Glucose	1·0 „
Water	1000 c.cm.

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[1] Saguchi's work assumes greater importance because of the recent discussion at the International Experimental Cytological Conference at Cambridge, where Dr. Canti declared that he could find no Golgi apparatus in the tissue cultured cells he had examined. Saguchi's paper is amply illustrated with microphotos of the Golgi apparatus.

The pH of this solution is circa 8·8. That of snail blood is circa 8·4. The laboratory Ringer previously used is 7·4. The preparations were put up between two square or long cover-slips, with a foot between them, and vaselined to a slide. They were kept on the bench at room temperature in petri dishes containing damp cotton-wool. The duration of such preparations is about five days, after which, owing to the enormous number of bacteria present, cessation of growth and clumping of amoebocytes takes place.

We have to thank Mr. Douglas Glen for taking the excellent photographs with which this paper is illustrated.

Cytology of Mantle Cavity Wall.

The histology of the mantle cavity wall has been already referred to in a previous paper by Gatenby and Duthie (5), to which the reader may be referred. It remains for us to describe the cytology of those cells which are most prominent in outgrowths from ex-plants of the mantle cavity wall. Text-fig. 1 is drawn from a section of a Weigl (Mann-Kopsch) preparation of the mantle wall. The Golgi apparatus alone is shown, and categories of cells which grow out on the cover-slip are, (*a*) the shell (mantle epithelium) at (ME), the amoebocytes (A), and the pulmonary cavity epithelial cells (PE). Other elements depicted but which do not grow out on the cover-slip are pigment cells of Leydig (P) ganglion cells (GA), and muscles (M).

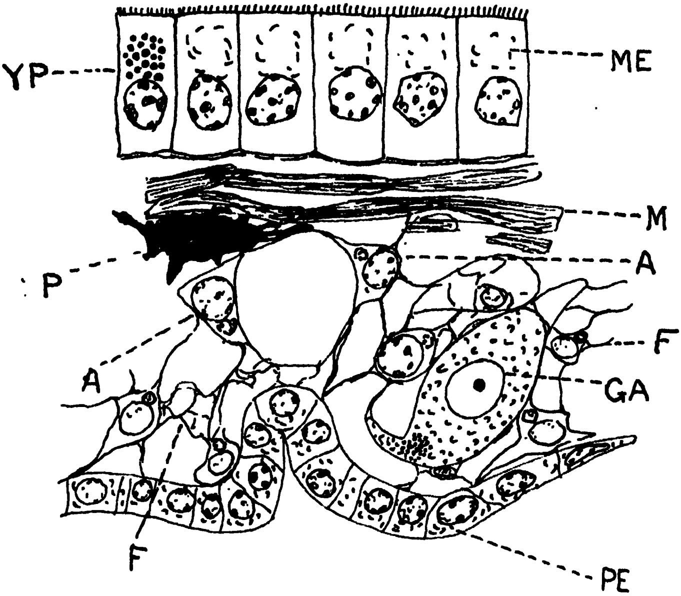
Now if sections of the mantle wall fixed in corrosive sublimate acetic acid and stained in Mann's methyl blue eosin be examined, it will be found that the connective tissue network of the wall is formed of stellate elements. That is to say, that the amoebocytes form the connective tissue, which is identical with the amoeboid elements which so freely emigrate from ex-plants. This is a point of some importance to those interested in the tissue culture of vertebrates. The blue staining fibrils are actually pseudopodia of the amoebocytes, and the network structure seen in tissue cultures of Helix, such as that in fig. 6, Pl. 21, is actually the normal arrangement of these elements in the mantle wall tissue. We do not at present believe that there are two categories of cells, one connective tissue, another amoebocyte.

In view of the fact that the amoebocytes are actively phagocytic this point is worthy of note.

Cut across the middle, one of these amoebocytes is shown in fig. 17, Pl. 22. The cell has been impregnated by Kolatchew's method, and both mitochondria and Golgi apparatus can be made out; the mitochondria are few and granular, while the Golgi apparatus is a sphere covered by dictyosomes which blacken, and whose individual parts are usually ill-defined. In the corrosive methyl blue eosin preparations mentioned above, the cytoplasm of such cells is blue, the nucleus purplish red. Now so far as we are able to say, in every case such cells contain a Golgi apparatus. The importance of this will appear later.

The mantle or shell epithelium is cytologically a very remarkable one. With corrosive sublimate acetic or Bouin's fluid, each cell is found to contain a number of bright yellowish brown pigmented granules, which resist fat solvents. Such a cell is inserted in Text-fig. 1, at YP, for comparison with the Kopsch cells nearby. Now in Helix aspersa the granules, as we have said, are very definitely pigmented, but in the Roman snail, Helix pomatia, the granules though present, and visible in the living, are not pigmented in the white specimens of this species. If these cells be examined in the living condition the granules appear to be principally of the pigmented variety, but hyaline ones are to be seen as well, as shown in fig. 11, Pl. 22, pigmented (GR), non-pigmented (D). Now in the average Weigl (Mann-Kopsch) or Da Fano preparation, the granules all go quite black, but by examining other parts of the epithelium, especially in the osmic preparations, the position of the granules may be seen to be occupied by unstained spaces, but surrounding the outer area of these spaces very typical osmiophile dictyosomes appear. This appearance is indicated in Text-fig. 1, ME. Turning now to fig. 12, Pl. 22, we see an intermediate condition in which a certain number of the granules are found to be partly embraced by osmiophile dictyosomes. Our view is that the pigment granules are formed directly in connexion with the Golgi apparatus, and by some change in the chromophobe part of the latter which becomes loaded with pigment.

Another cell category which may now be noticed is the pulmonary epithelial cell, of which a 'Kopsched' specimen is drawn in fig. 18, Pl. 22, and a part of the epithelium in Text-fig. 1, PE. Now these cells contain large numbers of spaced granules, which are refractive but non-pigmented in the living state. Examined after the Weigl method, numerous dictyosomes are found scattered among the pale granules, the latter not being osmiophile. No connexion could be traced between Golgi elements and pale granules. Before discussing the structure of the sparsely scattered ganglion cells which occur in the mantle wall, the reader's attention may be called to fig. 8, Pl. 22, in which the well-known Helix spermatocyte is once again given for comparison with the other cell elements already mentioned. Here



TEXT-FIG. 1.

Mann-Kopsch (Weigl) section of normal mantle cavity wall showing mantle epithelium (ME), muscle (M), pigment cell of Leydig (P), amoebocytes (A), spun-out amoebocyte fibrils (F), ganglion cell (GA), pulmonary cavity epithelium (PE). The Golgi dictyosomes are shown as curved lines. At YP the cell depicted is from a corrosive sublimate acetic preparation.

we have the box-like arrangement of dictyosomes embracing an archoplasm or chromophobe area. At mitosis the dictyosomes separate, each with its chromophobe part, and pass to the asters.

Finally we pass on to the ganglion cells, of which one is drawn in Text-fig. 1, at GA.

In view of the papers of Parat (**11**) and Young (**14**) on molluscan neurones, we have taken considerable trouble in going over our previous results. In the first place instead of using Ringer exclusively, we have employed Hédon-Fleig solution. This solution is most favourable to Helix cells. The cephalic ganglia have not been used as they are not small enough to allow examination without a good deal of manipulation. Just under the region where the salivary duct joins the buccal mass are two small swellings, the buccal ganglia, which can be dissected off without much handling. These are just transparent enough to enable the neurones to be examined through the dense sheath of fatty and other cells surrounding the ganglia.

In such ganglia the neurones examined immediately after extirpation are found to contain two clearly marked categories of granules. Brownish pigmented granules, usually, but not always, heaped into the axone region, and non-pigmented but very refractive granules spread throughout the cytoplasm. From their disposition and number it is now believed that they are the Golgi bodies. Supra-vitally they do not show a demarcation into two parts, dictyosome and chromophobe area, but in fixed preparations such demarcation can be seen quite easily in every Golgi body. This view of the nature of these granules is at variance with our previous conclusion (**2**), and also with the recent work of Monné (**10**) who thinks the Golgi bodies are invisible in the neurone, intra vitam.

Young claims that the very large globules or granules which stain avidly in neutral red can be seen intra vitam in squid neurones. This, therefore, at once proves that the neutral-red 'vacuome' so far as it is represented by these globules is not a segregation artifact, but is due to the higher solubility of neutral red in the globules than in the surrounding cytoplasm. In addition Young describes a number of much smaller granules which go black in osmic acid after treatment with chrome, and which he appears to have wrongly identified as the Golgi apparatus.

At all events it is certain that in cephalopods the neurones contain three categories of bodies, namely, the Golgi bodies, not demonstrated by Young, the 'vacuome' or globules which take up neutral red, and the lipochrome granules of Legendre, which are usually in the axone. The lipochrome granules of Legendre appear to correspond to the 'Nissl granules' of Young. Through the courtesy of Professor Victor Grégoire of Louvain, the senior writer has been able to examine properly prepared Kolatchew sections of squid neurones, in which the Golgi bodies were as already described in Helix (**2**, **6**, **10**), except a little larger, in proportion to the size of the cell.

In Text-fig. 2 is a diagram of the various categories of bodies present in molluscan neurones according to Young and the present writers. Above the line are the structures we are prepared to recognize at present, namely, the lipochrome granules (LG), the mitochondria (M), the Golgi bodies (GB), Nissl substance[1] (NS), and certain globules (NR) of unknown nature and function which happen to take up neutral red. They are probably storage bodies of a lipin nature. Below the line are the bodies recognized by Young, namely, the same neutral-red bodies (NR), granules which he calls Golgi bodies (GB) which we believe are not Golgi bodies, mitochondria (M), and an area of Nissl substance (NS). It will be noted that neither the Golgi apparatus nor the lipochrome bodies have been recognized by Young, but were previously noted by Legendre, Kolatchew, Brambell, Gatenby, Parat, and Monné.

So far as Helix aspersa neurones are concerned we believe that the following bodies are certainly demonstrable:

(*a*) The Golgi apparatus consisting of perfectly typical dictyosomes with chromophile and chromophobe parts.

(*b*) The mitochondria.

(*c*) The lipochrome granules of Legendre (**8**).

If the neurones are placed in neutral red, in many cases red granules or globules appear in the cells. We are unable to say whether they are artificial or not, but we consider that neither in size nor distribution are they identical with the dictyosomes of the Golgi apparatus.

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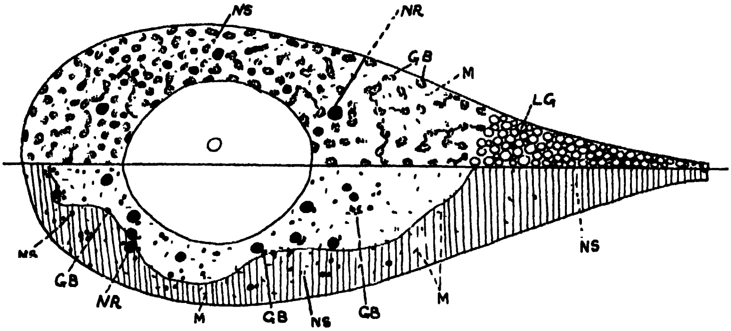
[1] We have made some Nissl preparations of snail neurones, and have found some doubtful peripheral granules which might be homologous with the real Nissl substance of vertebrate cells.

It is obvious that the pulmonate is not such favourable material for study as the cephalopod neurone; for, if granules other than the Golgi bodies and lipochrome granules exist in Helix, they are not obviously so large as to be plainly visible in living cells like those described by Young in cephalopods.



Text-fig. 2.

Diagram of substances present in neurones of Helix and Cephalopods. Below the line according to Young (14). The Nissl substance area is represented by vertical lines. A circumnuclear space is left in the middle in which the 'Vacuome' or neutral-red globules (NR) and the 'Golgi bodies' (GB) are practically exclusively situated. Above the line is the neurone according to the present authors. The true Golgi apparatus is a very large and conspicuous system of dictyosomes (GB) with chromophile and chromophobe parts. At LG are the lipochrome granules which in some of Young's figures are marked Nissl substance. The large globules (NR) have not been found in Helix, but Young's account of them is accepted. The mitochondria (M) are scattered in both halves of the neurone depicted. Except for the absence of Golgi bodies in the axon, no zoning could be recognized in Helix neurones.

THE GOLGI APPARATUS OF THE PULMONATE MOLLUSC.

A typical Kolatchew or Weigl prepared neurone is shown in fig. 15, Pl. 22, except for the granules GR, which are hard to find in any but atypically impregnated neurones. Now as previously stated by the senior writer and Brambell (**2**), 'the Golgi apparatus consists of rod or ring-shaped dictyosomes (D) each enclosing a chromophobe archoplasm'. Type dictyosomes are shown in fig. 16, Pl. 22, from an osmicated cell on the left, and from a formalin silvered cell on the right. In Da Fano and Cajal preparations the dictyosomes look more granular and less scale-like or batonette shaped. Moreover, in formalin silver preparations, as is the almost universal experience of cytologists, the Golgi bodies are clearer cut, and less likely to be confused with mitochondria.

It will now be clear that the present writers regard the dictyosome (chromophile-chromophobe) bodies, such as are found in spermatogenesis (fig. 8, Pl. 22), and oogenesis, and universally in Helix in somatic cells (figs. 7, 12, and 13, &c., Pl. 22) as the Golgi bodies. Other granules, which do not have the chromophile-chromophobe parts, and are not crescentic or scale-like, are not the Golgi bodies.

Now in the living neurones the very large granules depicted by Young, and shown in Text-fig. 2, at NR, do not appear in Helix. We cannot state definitely that they are not present, but they cannot be seen as refringent globules in any neurones we have examined—and this is all we can say. Moreover, in successful Golgi preparations made by both silver and osmic methods no such granules are demonstrable. In some parts of our preparations atypically impregnated additional granules are seen, and are shown in fig. 15, Pl. 22, GR. These are not Golgi bodies, are not definitely distributed in the neurone, but may possibly be the same as Young's so-called Golgi bodies in Cephalopod neurones.

The reader interested in this problem may profitably consult the admirable work of L. Monné (**10**) of the Lwow School, who gives a masterly description of Golgi bodies and neutral-red globules in various types of cells in Helix, Cerithium, and Paludina. We wish to make it clear, however, that we recognize no such cell organ as the 'vacuome' and have no knowledge of any such structure. By some observers the word is used for convenience for a variety of secretion or segregation granules known for thirty or forty years past.

OUTGROWTHS FROM EX-PLANTS.

On Plates 19–21 are a number of microphotographs which show the character of the outgrowing cells. Fig. 1, Pl. 19, gives a low-power view of a 24-hour preparation in Hédon Fleig medium. With the exception of two areas (XY) on the left middle, and the left bottom parts of the ex-plant, cells are migrating out. everywhere. Many amoebocytes have wandered far away from the ex-plant, examples being marked F. Already the nature of the outgrowths is somewhat different in parts. For example at X, and below (above the letters XY) the cells look thicker and less hyaline than in the area at Z. The reason for this is that at the last-mentioned area the amoebocytes have begun to stretch out and form a connected network system, whereas at the region X the cells are actively migratory and less stretched.

In fig. 2, Pl. 19, the process of migration and organization into a network has gone on, and the outgrowth in places 2 millimetres broad can be seen with the naked eye as a whitish area around the black ex-plant. Cells are still migrating outwards as at X, but the tendency is for the outgrowth to keep a united front. The amoebocytes tend to form large spaces (S) in the network. By this stage outward migration of the mantle (shell) epithelial cells (Text-fig. 1, ME), along the immediate edge of the ex-plant (ME). These elements are not mixed freely with the amoebocytes, but tend to stick to the cover-slip nearest the mantle epithelium.

In the square outlined in fig 2, Pl. 19, we have an area which is shown at a higher power in fig. 5, Pl. 21. The outgrowing front (F), of the amoebocyte network is shown, and here and there (F) separate amoebocytes are still wandering.

We now proceed to the period of 3 to 5 days when the bacteria begin to increase enormously. Hitherto rapidly moving bacteria as well as types which appear immobile have been easily found at all stages, but by 4 or 5 days these bacteria become so numerous that the liquid in which the preparation lies is swarming with them. In fig. 3, Pl. 20, which is from a living preparation, the bacteria are seen as minute white dots (B). A similar condition is present in fig. 6, Pl. 21, but only in the lower part of the photograph, the region immediately next the ex-plant not yet being seriously infected. Now fig. 6, Pl. 21, at 5 days, is still an active preparation, but is at the beginning of the end, for clumping of the amoebocytes has begun. This clumping always occurs in preparations which for some reason or other are dying. It may happen in 12- or 24-hour preparations and ultimately results in the formation of more or less isolated aggregations, in which delicate networks are fewer, and many rounded amoebocytes are present.

In our first Ringer preparations the clumping took place from 24 hours to 3 days, but the use of Hédon Fleig saline enabled the ex-plants to flourish 2 or 3 days longer. That is to say, the bactericidal ability of the cells of the ex-plant was greater in the more suitable medium, as might be expected.

In fig. 6, Pl. 21, part of the preparation still shows the normal amoebocyte network, whereas at c the clumping is marked. The more refractive elements in the right upper corner of the preparation are, we believe, amoebocytes which are choked with bacteria and are dying or dead. More advanced and typical clumps, which must contain many hundreds of cells, are shown in figs. 3 and 4, Pl. 20. Fig. 3 is from a living preparation, fig. 4 from one fixed in Bouin, and stained in Ehrlich's haematoxylin. Practically all the cells in the outer fringe of the clump contain included bodies (I) which they have engulfed. Now it will be noted that the large clump in fig. 4 was produced after only 24 hours (pH 7·4), whereas that in fig. 3 was in a much less necrotic state after 5 days (pH 8·4). There is a group of dying cells at K, which have rounded off, after phagocytosing as many bacteria and detritus as possible.

THE AMOEBOCYTE NETWORK IN FIXED PREPARATIONS.

If the network of tissue cultures be fixed in sublimate and stained in Mann's methyl blue eosin, it takes the blue coloration (that is with our present specimen of the stain) in exactly the same manner as the network in the control sections of normal mantle wall, thus reinforcing our opinion that the outgrown cells are actually the connective elements of the tissues.

SUPRA-VITAL STAINING.

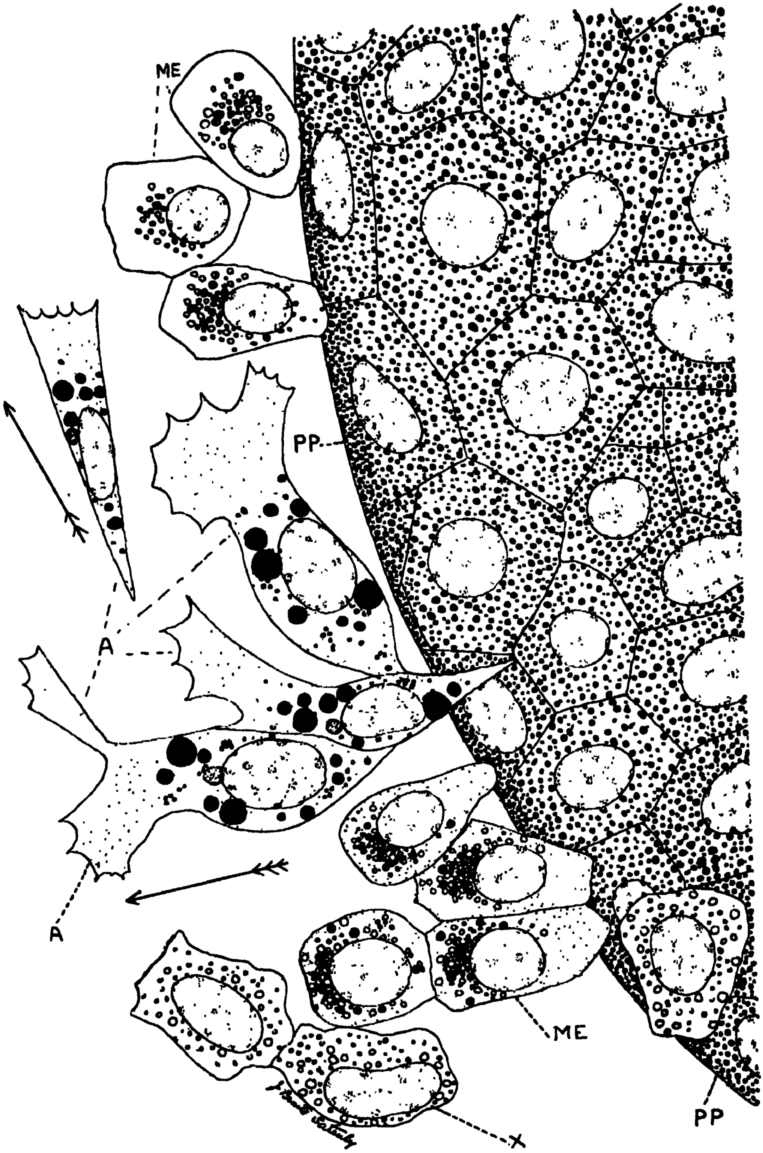
We used neutral red (Grübler, National Aniline Corporation of America), Janus green, and Dahlia violet.

The ex-plants and their outgrowths may be stained in one of two ways. Either the stain may be mixed with the Hédon Fleig saline, or after outgrowth has taken place in the untinted saline; this may be drained off, and the coloured saline drawn over the tissue cultures. In any case the amoebocytes are the first to stain and absorb large quantities of the stains tried. They become so filled with neutral red and Dahlia violet that they ultimately may disintegrate.

We have used spermatocytes as a control on our solutions. We have found that in Hédon Fleig saline, these cells stain in weak solutions in 2 hours as shown in fig. 9, Pl. 22, and like fig. 10, Pl. 22, in 4 to 6 hours. The unstained cell is depicted in fig. 8, Pl. 22. In Ringer of an unsuitable pH, comparable concentrations of the stain do not act so quickly or surely. The Ringer solution is pinkish, the Hédon Fleig yellowish, though inside the cells the stain is bright cherry red.

In Text-fig. 3 is the edge of an ex-plant with four categories of cells, showing the relative avidity with which the neutral red is taken up. The amoebocytes speedily fill themselves with large globules, and form by far the most conspicuously stained units. The pulmonary epithelial cells come next (PP), but we could never satisfactorily stain the outwandering mantle epithelial cells (ME). Even after some days in Ringer or Hédon Fleig saline and neutral red, the mantle cells were empty of stained granules. In some cases, however, a few granules were stained, especially outside the Golgi apparatus region. It may be stated, however, that the Golgi region does not segregate stain like that of the spermatocytes. Now in fig. 11, Pl. 22, we have a drawing of a living cell, with stained granules at NR.

Text-fig. 3. Part of an ex-plant edge a few hours after mounting in neutral-red Ringer, the cells in contact with or wandering on the cover-slip alone shown. These consist of four types—amoebocytes (A), mantle epithelial cells (ME) which have not stained, pulmonary cavity epithelium (PP), and certain other cells (X) of unknown nature, which have not stained. The granules in the mantle cells (ME) are mainly yellowish pigment and not neutral red.



TEXT-FIG. 3.

In figs. 13 and 14, Pl. 22, we have two pulmonary epithelial cells, one after Weigl's osmic method, another in fig. 14, after staining in neutral red. The non-osmiophile granules, present intra vitam, take up the neutral red very energetically. After some time some of the neutral-red granules get larger, and ultimately very large globules are present. Here again we have a type of reaction to neutral red, quite different from that shown in figs. 8–10, Pl. 22.

In fig. 7, Pl. 22, is an amoebocyte, with Golgi apparatus at G, and many neutral-red granules. We have gone carefully into the question of the staining and structure of these cells. Some of these cells appear to contain globules other than mitochondria (M) and Golgi apparatus, after they wander out, but we are not sure whether these are new acquisitions or not. At all events the neutral-red globules seem to be able to appear as definitely non-pre-existent granules anywhere in the cytoplasm. It is a question whether the Golgi apparatus begins first by absorbing the neutral red. We believe that in most cases the segregation does not begin in a definite situation in each amoebocyte which might correspond with the position of the Golgi apparatus, but we cannot yet be sure, and are at present investigating this question. As to whether the Golgi region is the first to take the stain or not we can say this much—the neutral-red globules appear subsequently everywhere throughout the cytoplasm. Again we wish to point out how different is this reaction from what occurs in the spermatocytes depicted in figs. 8–10, Pl. 22.

We should also like to mention that L. Monné (**10**) gives a figure of a connective tissue element with both neutral-red granules and Golgi apparatus separate, the latter not stained by the neutral red.

DISCUSSION.

Molluscan Golgi Apparatus.

According to Young (**14**) the globules which take up neutral red can be seen in the unstained and living neurones of cephalopods. If this observation is correct it shows that the neutral-red globules are not all segregation artifacts in neurones. We believe that some of them may be segregation artifacts as in Helix spermatocytes, where they go on increasing in number and growing in size according to the period of exposure of the cells in the dye. Young also claims that the neutral-red globules lie in a somewhat restricted area of the cytoplasm, but except for the root of the axon this zoning does not apply to the Golgi elements which are scattered everywhere.

In his valuable study Monné (1930) states that 'Im einfachsten Fall ist das Golgi-Apparat-Element eine schwach gewölbte, bikonvexe Linse, welche an ihrer Kante von einem ringförmigen Falz eingefasst ist'. Of the 'vacuome' he says, 'Das Vacuome-Element scheint bläschenförmig zu sein'.

It will be clear that the two structures are quite different.

The 'Zône de Golgi'.

According to Parat (**11**), the cell contains a 'zône de Golgi', consisting of dictyosomes associated with neutral-red globules. We admit that in the case of the spermatocytes of Helix, it is quite possible to manufacture a completely convincing 'zône de Golgi' by leaving the cells to segregate the neutral red as in figs. 8–10, Pl. 22. The normal cell is shown in fig. 8, and it has no cavities which impregnate with silver or osmic Golgi methods. The Golgi apparatus of this cell is the dictyosomes, and nothing else.

Turning to the other cells we have studied, we find that the shell or mantle epithelial cells refuse to take up neutral red even when neighbouring amoebocytes and pulmonary epithelial cells are crammed with granules of neutral red. It is evident that these cells have a Golgi apparatus but no 'zône de Golgi'. When we examine the behaviour of the pulmonary epithelial cells we find that the already existing granules quickly absorb neutral red. These granules have no relation with the Golgi dictyosomes.

In all these cells the only regularly occurring and homologous bodies are the argentophile and osmiophile dictyosomes and the mitochondria. It is not possible to compare the reactions of each cell to neutral red, or to identify with certainty a system or zone associated directly with the dictyosomes. We believe that in certain cells, like the spermatocyte, there may be a special region or kind of cytoplasm in which aggregation of the neutral red takes place, and that during mitosis this region is definitely distributed. Whether this is the chromophobe part of the Golgi apparatus in Helix aspersa spermatocytes we do not know.

Tissue Culture.

The most interesting point about this form of tissue culture is the continuous outgrowth for periods up to 5 days, in the presence of large and ever increasing bacterial infections. We are at present trying to destroy the bacteria by running off the Hédon Fleig saline and replacing for a time by blood or solutions of epidermal cells, but our experiments have not gone far enough to enable us to give data. It is hoped to get much longer growths by using the natural antibodies of the snail.[1] In any case the method is now reliable enough to give interesting material for research on living cells in vitro, without the complication of a rigid surgical asepsis.

The proper evaluation of our results can best be made by those who have had long experience with vertebrate tissue cultures. We are not, for instance, yet quite sure as to the nature of the amoebocyte. Monné's figures of the connective tissue cells of Helix lutescens and pomatia closely resemble what we consider to be the amoebocytes (connective elements) of the mantle cavity wall, and we have little doubt that subsequent workers in this problem will agree that our amoebocytes are actually the connective elements of the Helix tissues. We find that the connective elements in situ absorb neutral red just like the outwandering amoebocytes.

Reviewing the questions which arise with regard to the migrations of cells in vertebrate cultures, Craciun (1) says: 'Le foie, par exemple, produit en culture pendant les deux premiers jours des cellules amiboïdes isolées: neutrophiles, lymphocytes, macrophages. Plus tard apparaissent des éléments qui tendent à former des tissus continus: épithélium, endothélium vasculaire, mésothélium péritonéal, fibroblastes.'

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[1] More recently Miss Hill and Mr. J. Macdougald have succeeded in getting aseptic preparations by using a mercury vapour lamp, and by careful sterilization of slides, &c., but so far the growths show little improvement on our previous results.

Possibly the behaviour of ex-plants from the snail bears some resemblance to this. Some of the first smaller outwardly migratory elements may be blood-corpuscles, which are later followed by the tissue which forms a continuous network, and subsequently by the slowly moving epithelial cells.

We have often put up ex-plants in large quantities of blood in hollow-ground slides, and found that after many days bacteria appeared to be absent. In no case did we ever end up with the thick milky suspensions of bacteria, so common in saline or thin double cover-slip blood preparations. Nevertheless these apparently sterile ex-plants did not grow out appreciably, and as we remarked in the introduction we do not yet know why this is so.

There are a few further points of interest which we may mention here, and which we are still trying to elucidate. For instance, we have already mentioned (5) that we believe that the amoebocytes multiply by amitosis. In all the luxuriant outgrowths now obtained by improved methods not one mitosis has been found, but we have many preparations showing what we both consider to be amitotic divisions. This will be dealt with elsewhere, when our evidence has been properly collected, but we may mention that in some amoebocytes, in what the senior author considers to be faultless Da Fano impregnations, the Golgi apparatus is absent.[1] We believe that during the rapid amitotic divisions of the amoebocytes the sphere and its Golgi material go over whole to one daughter cell. We have yet to find amoebocytes which cannot segregate neutral red in large quantities, and at present we hold the view that an amoebocyte can live and function without the presence of a Golgi apparatus. We have no observations on the centrosome at present to offer.

SUMMARY.

1. By keeping pieces of mantle cavity wall in Hédon Fleig saline it is possible to make cultures which grow out for about

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[1] Saguchi (op. cit.) also finds that tissue-cultured cells have no dictyokinesis, though they start out normal, that is to say, with a Golgi apparatus. Saguchi's paper is of much value to all those interested in the Golgi apparatus of tissue cultured cells.

five days. After that time the bacteria have increased so enormously that growth is checked, though the cultures will live for several weeks longer in a suspended condition.

2. The main type of cell which grows out is an amoeboid element, identical, it is believed, with the general connective elements of the normal tissue of the snail.

3. Neutral red stains, is segregated, or is deposited in the various categories of cells, in various ways. For example, in spermatocytes it appears almost always inside the Golgi apparatus, thus forming with the dictyosomes a 'zône de Golgi' of Parat. In the pulmonary epithelial cells the neutral red at once stains the pre-formed granules which are visible in unstained living cells. These granules are not directly related to the Golgi bodies. In amoebocytes the neutral red appears principally as large segregated globules, anywhere in the cytoplasm. Finally, into the mantle epithelial cells it is difficult to get neutral red, when other cells are already well stained.

4. The only homologous bodies in the cytoplasm of these various categories of cells are the Golgi bodies and mitochondria.

5. In no case has a mitotic figure been found in any Helix culture. Cells suggesting division by amitosis are commonest when the cultures are growing out fastest.

DESCRIPTION OF PLATES 19 TO 22.

The photographs were taken with a Leitz mikam camera, on a ¼ plate (Panchromatic). They were then enlarged twice with a Kodak enlarger, and were finally reduced to the size which they now are.

The scale is given in close proximity to each photograph.

Plate 19.

Fig. 1.—General view of ex-plant (24 hours).

Fig. 2.—Enlarged view of outgrowth (2 days). The part enclosed in the square is shown in fig. 5, much enlarged.

Plate 20.

Fig. 3.—After 5 days, bacteria very dense.

Fig. 4.—This is a fixed and stained preparation, after 24 hours Ringer, pH, circa 7.

PLATE 21.

Fig. 5.—Part enclosed in square in fig. 2, much enlarged.

Fig. 6.—Preparation (living) after 5 days and still growing well, but starting to clump.

PLATE 22.

Fig. 7.—Amoebocyte staining in neutral red.

Figs. 8–10.—Normal and staining spermatocytes.

Figs. 11 and 12.—Mantle epithelium cells, first in fresh state, second by Weigl method.

Figs. 13, 14.—Pulmonary epithelium cells, first by Weigl, second by neutral red, fresh.

Fig. 15.—Helix aspersa neurone, Weigl method.

Fig. 16.—Dictyosomes, first osmic, second silver methods.

FIG. 17.—Amoebocyte (connective tissue) from tissue as it is in situ, but processes not shown.

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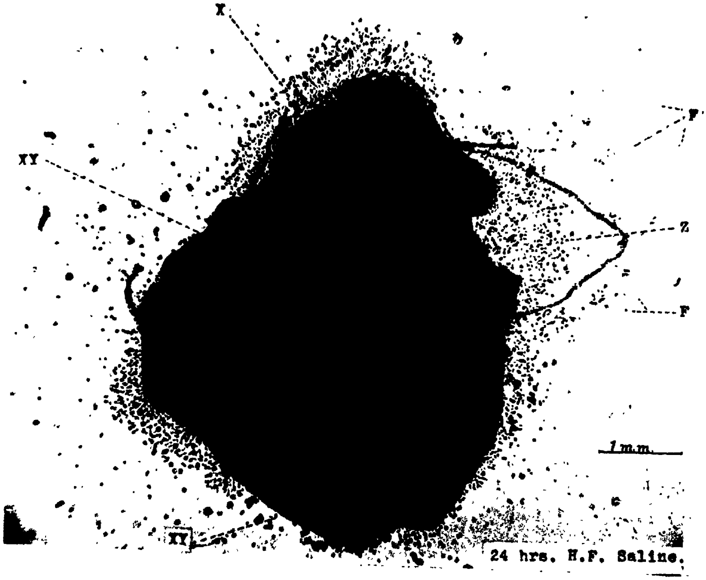

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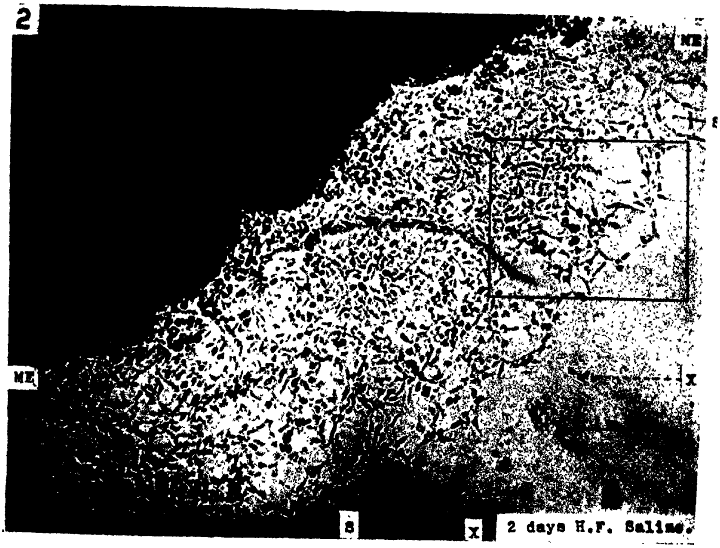

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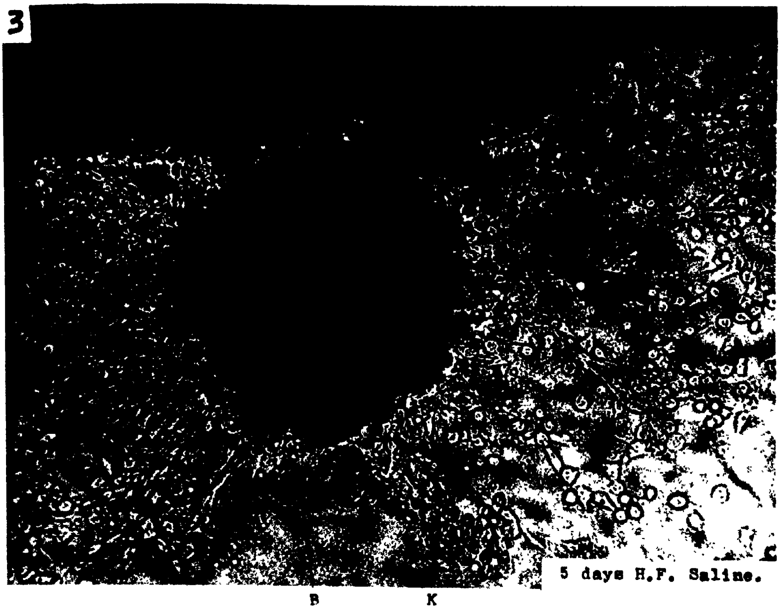

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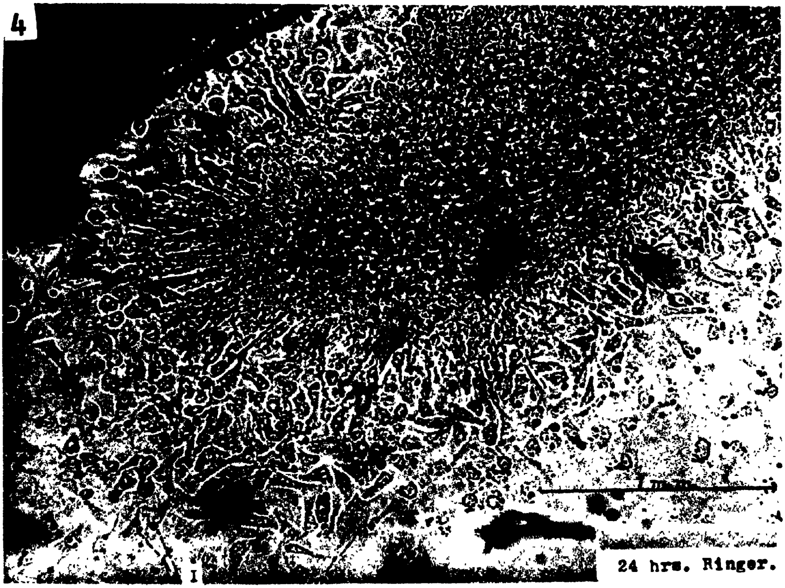

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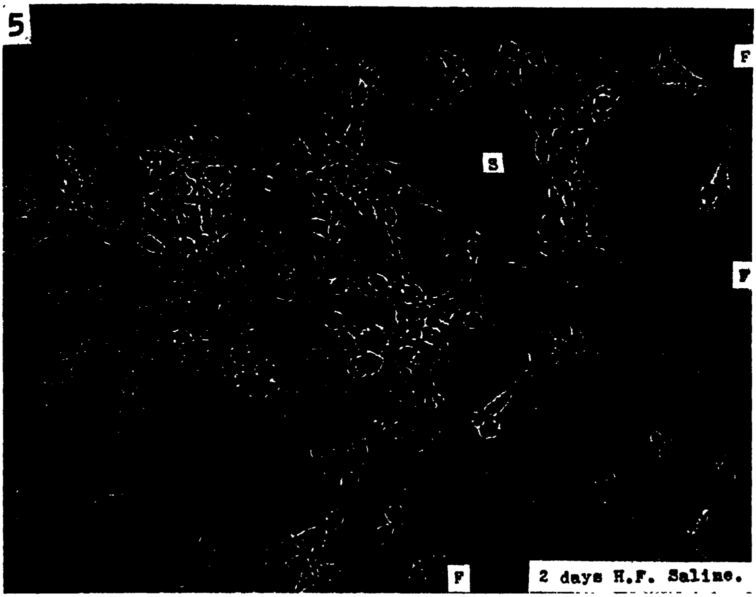

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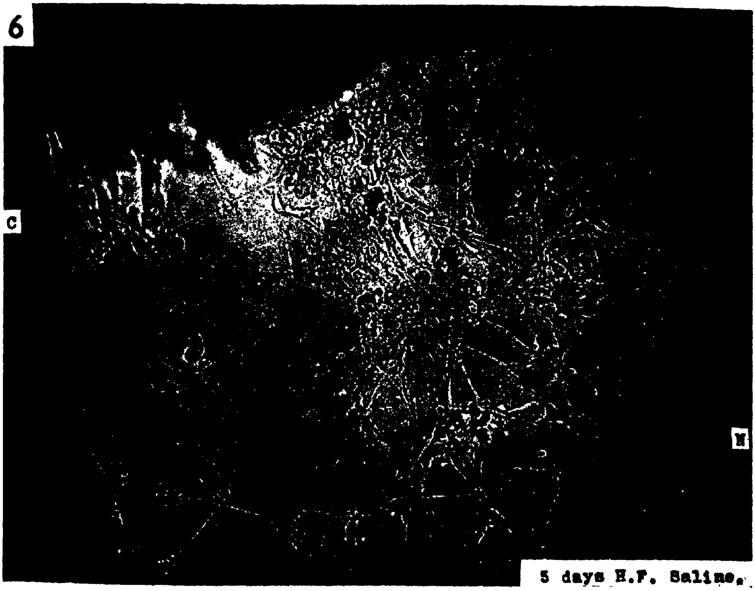

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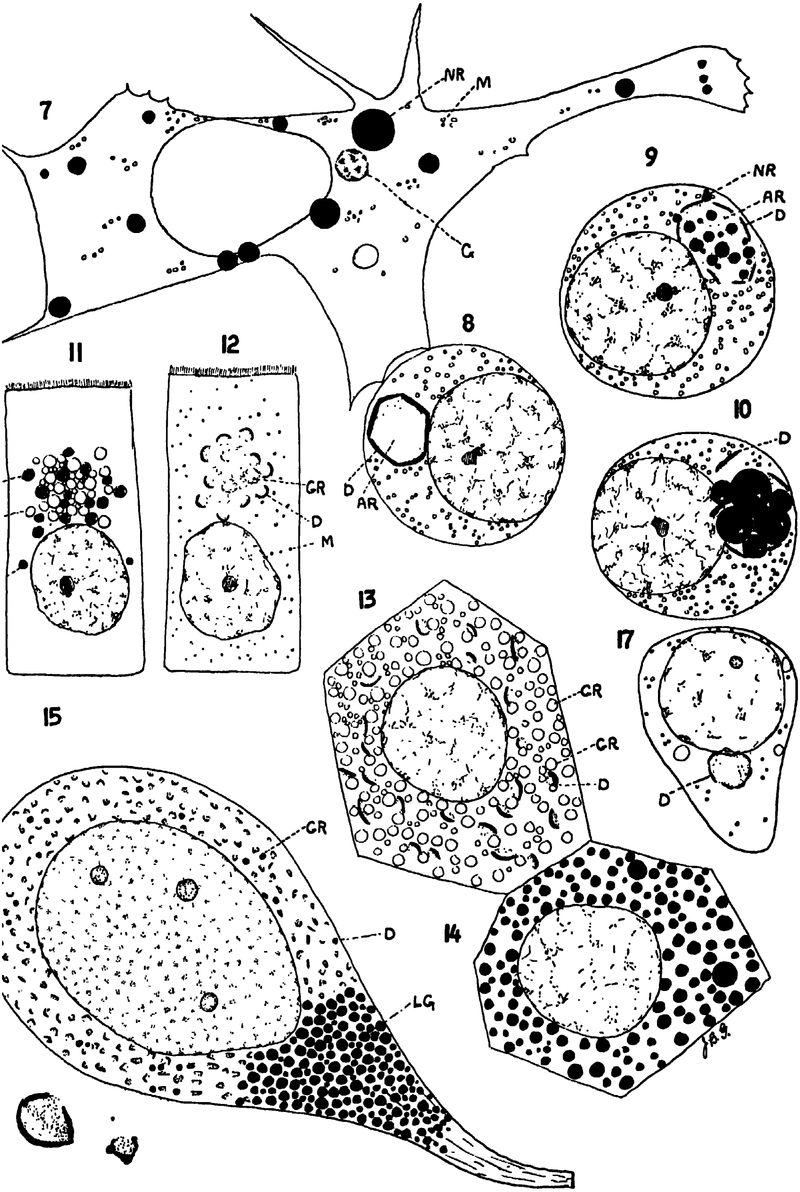
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La Spermatogenèse et l'Ovogenèse du Mallophage Goniodes stylifer

By

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With Plates 23 to 25 and 5 Text-figures.

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INTRODUCTION.

Doncaster et Cannon, dans leur travail sur la spermatogenèse de Pediculus humanus, sans arriver à des conclusions précises, posèrent un problème du plus haut intérêt concernant le cycle chromosomique du Poux.

Les cellules diploïdes isolées, que l'on trouve dans la région goniale du testicule au milieu des spermatogonies de taille plus réduite, ne sont, pour les auteurs anglais, que des éléments somatiques. Les gonies elles-mêmes, qui montrent un nombre réduit de chromosomes, seraient pourtant diploïdes en fait, l'haploïdie ne résultant que d'un accouplement précoce des éléments des paires chromosomiques, phénomène prosyndétique se passant à une époque très reculée dans l'évolution du testicule et décelable par la forme allongée des chromosomes à la métaphase et par leur structure dyadique à la prophase.

Doncaster et Cannon ne donnent pas d'explication au sujet de la seule et unique division cytaire hétéropolaire, semblable à la seconde mitose de réduction de l'Abeille, mais ils supposent qu'il doit y avoir un rapport entre elle et la pseudo-haploïdie des spermatogonies.

Bien que le beau travail de Ries, sur l'ovogenèse des Mallophages et des Pediculides, soit d'un intérêt primordial, puisque c'est le seul à peu près complet qui ait paru sur ce sujet, il n'apporte aucun fait pouvant trancher la question du cycle chromosomique au moins chez la femelle, car l'auteur n'a pas étudié le noyau des jeunes éléments germinaux.

Il faut du reste, pour résoudre un tel problème, faire une étude parallèle de la lignée germinale des deux sexes, et c'est ce que j'ai essayé de réaliser dans le présent travail.

C'est en 1930, à l'Université de Columbia (New York) et à l'instigation du Professeur F. Schrader, que j'ai commencé cette étude. J'obtins alors de fort belles préparations de spermatogenèse de Pediculus humanus.

De retour à Genève, je dus chercher un autre matériel, la difficulté de se procurer des Poux humains en assez grande quantité excluant toute recherche approfondie. J'eus la bonne fortune de trouver une espèce de Mallophage du Dindon (Meleagris gallopavo), le Goniodes stylifer (Nitzsch), dont la spermatogenèse suit, dans ses grandes lignes, celle du Pediculus.[1] Les éléments diploïdes mélangés aux éléments haploïdes goniaux, de même que l'unique division cytaire hétéropolaire, se retrouvent dans le testicule des deux espèces.

Ce Goniodes stylifer m'a permis de mettre au point une technique adéquate à l'étude de l'ovogenèse, parallèlement à celle de la spermatogenèse, et la plus grande partie de cette recherche était déjà achevée lorsque je pris connaissance du très beau travail de Ries, 'Die Prozesse der Eibildung und der Eiwachstum bei Pediculiden und Mallophagen'.

[1] Il en est de même dans la spermatogenèse d'un Docophorus, d'un Lipeurus et d'un Menopon, ce qui permet la généralisation des phénomènes décrits dans ce travail.

Il est regrettable que les éléments germinaux, mâles surtout, soient d'une si petite taille, car les recherches de ce fait ne peuvent être poussées au delà d'une certaine limite. Une méthode d'élevage cependant, permettrait de mettre un point final aux conclusions de ce travail.

Avant de commencer, je tiens à exprimer à Monsieur le Professeur Schrader ma grande reconnaissance pour tout l'intérêt qu'il m'a témoigné pendant mon séjour à Columbia. Mes remerciements vont aussi à Monsieur le Professeur Guyénot, dans le laboratoire duquel j'ai eu le privilège de continuer ces recherches, et à Monsieur André Naville, pour leur aide et leurs précieux conseils.

MATÉRIEL ET TECHNIQUE.

Matériel.

Dès qu'une Dinde a été tuée, je récolte toutes ses plumes dans un seau que je dispose sur un radiateur. Les Poux se dirigent vers la chaleur et sont facilement capturés dans la région inférieure du récipient. Au bout de trois à quatre jours, les insectes meurent, probablement plus à cause d'un défaut d'humidité que d'un défaut de nourriture. Il faut donc fixer les glandes génitales aussi vite que possible, car des processus dégénératifs s'observent très rapidement dans des ovaires ou testicules de Poux en captivité.

Le mâle adulte (stade IV) se distingue aisément de la femelle par son appendice copulateur chitinisé à la base de l'abdomen, mais il n'est pas possible de reconnaître les sexes des Goniodes qui n'ont pas atteint la puberté[1] (stade I, II, III).

TECHNIQUE.

Fixation.

Testicules.—Après essai des fixateurs cytologiques courants, tels que les liquides de Bouin, Allen, Carothers, Carnoy, Mann, Flemming, j'ai employé exclusivement ce dernier mélange qui est le seul donnant des résultats homogènes d'une finesse parfois remarquable.

Les testicules sont paires. Chacun d'eux est formé de deux ampoules se déversant dans un canal déférent. Chez l'adulte, il est facile de les extirper de la masse viscérale que l'on fait sortir par simple pression après déchirure de l'extrémité de l'abdomen. Il est évident que cette opération ne peut se faire que sous la loupe binoculaire. Les glandes mâles sont alors fixées 10 à 15 minutes dans le liquide de Flemming, lavées, déshydratées et éclaircies rapidement, puis coulées dans la paraffine après deux bains de 10 minutes.

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[1] Le Poux subit trois mues avant d'atteindre le stade adulte (IV).

Pour obtenir des préparations de testicules jeunes, des stades II et III, il est préférable de fixer l'abdomen entier. Après passage dans l'alcool à 70°, puis dans l'alcool butylique, on arrive à couper facilement pourvu que les bains de paraffine soient rapides.

Les éléments cellulaires étant fort petits, il faut couper à 4 microns.

Ovaires.—Si je n'ai pu déceler les testicules probablement minuscules, mais existants au stade I, j'ai pu par contre observer souvent des ovaires d'individus nouvellement sortis de l'œuf.

Il est nécessaire pour fixer les glandes génitales femelles des Poux des stades I et II, de fixer l'abdomen entier. Comme fixateur, les liquides de Carnoy, de Carothers et de Flemming surtout sont les meilleurs.

Pour l'étude des ovaires adultes, il est facile et préférable de les isoler avant de les fixer. Les extrémités des tubes ovariens ainsi que les ovocytes de moyenne taille, se coupent facilement[1] mais il est extrêmement difficile d'obtenir de bonnes préparations d'ovocytes prêts à être pondus et surtout des œufs. Leur coque est d'une résistance et d'une dureté qui excluent une bonne confection des coupes et l'on n'obtient le plus souvent que des lambeaux de tissu.

Le piquâge des œufs, permettant une pénétration aisée du fixateur, des déshydratants et de la paraffine, ne m'a pas permis d'obtenir de meilleurs résultats. Les coupes ont été faites à 5 microns.

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[1] L'alcool butylique, qui sert de déshydratant et d'éclaircissant, est très favorable au microtomage des objets durs comme certains ovaires d'insectes, des Poux en particulier. On évite ainsi, et les alcools forts, et les milieux intermédiaires.

J'ajouterai que la technique au carmin acétique sur l'animal frais m'a été de grande utilité pour comprendre la structure anatomique des ovaires. Cette méthode m'a permis aussi de numérer plusieurs cinèses goniales femelles.

Coloration.—La méthode de base a toujours été la coloration à l'hématoxyline de Heidenhain contrôlée par celle à la fuchsine sulfureuse de Feulgen-vert lumière.

Pour obtenir une bonne définition d'objets très petits (cellules du testicule et ovaire jeune) la coloration à l'hématoxyline s'impose, mais celle de Feulgen est des plus utiles pour l'étude des noyaux d'ovocytes avancés bourrés de deutoplasme, qui, coloré par l'hématoxyline, masque la vésicule nucléaire.

L'OVOGENÈSE DE GONIODES STYLIFER.

Description des ovaires.

Voici une brève description des ovaires basée sur des préparations au carmin acétique.

Les deux ovaires sont chacun formés de cinq tubes ovariens polytrophes.[1]

Au stade adulte (fig. 1 du texte, A) le filament terminal, qui ne contient que trois à quatre noyaux à sa base, est nettement séparé de la chambre terminale. Cette dernière renferme encore quelques éléments germinaux jeunes, mais la plupart d'entre eux sont en dégénérescence.

Dans un tube ovarien, on ne rencontre pas plus de quatre ou cinq ovocytes en évolution et qui parviendront à la maturité, ce qui amène une ponte d'environ quarante à cinquante œufs.

Tous les espaces de la chambre terminale, libres d'éléments germinaux, sont remplis de bactéries qui s'infiltrent dans les moindres interstices.

Chaque chambre à œuf est constituée par l'ovocyte en avant,[2] les cinq cellules nourricières en arrière, et par une enveloppe de cellules folliculaires surtout nette autour de la cellule germinale.

Dans une chambre plus évoluée, les cellules nourricières qui

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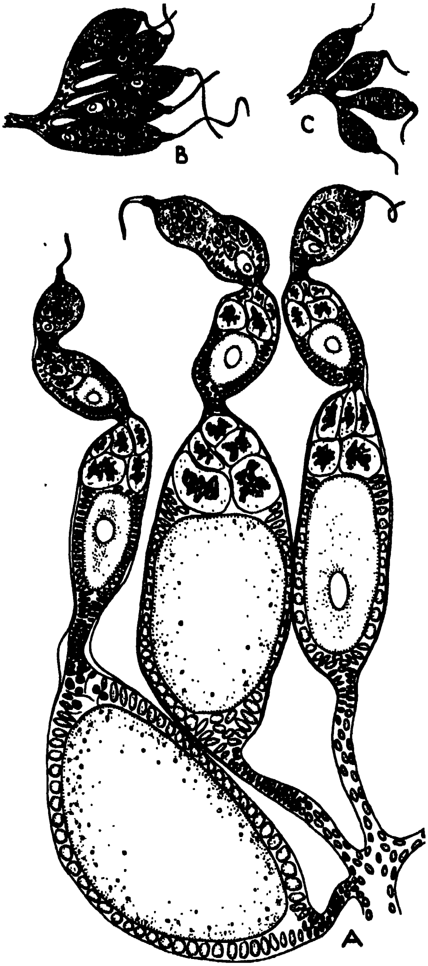
[1] Ce nombre peut varier dans de faibles proportions. On trouve fréquemment par exemple quatre tubes ovariens.

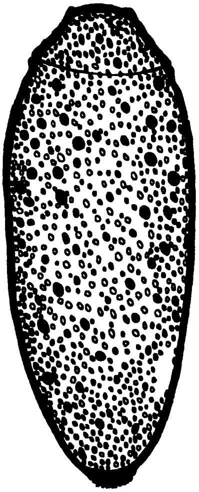
[2] En avant par rapport à l'embouchure de l'ovariole dans l'oviducte, et non pas par rapport à l'animal lui-même.



TEXT-FIG. 1.

A. Tubes ovariens de femelle adulte de Goniodes stylifer. B. Tubes ovariens de femelle au début du stade III. C. Tubes ovariens de jeune femelle au stade II. Carmin acétique. Gross.: 105.

ont terminé leur rôle, sont en voie de résorption. Les cellules folliculaires sont grandes et vont secréter la coque de l'œuf qui donne à ce dernier une si forte résistance au rasoir. Quant à l'ovocyte lui-même, il est rempli d'un deutoplasme abondant et atteindra bientôt l'aspect représenté à la fig. 2 du texte, qui représente un œuf mûr. A l'extrémité inférieure de l'œuf se trouve le micropyle. A l'autre pôle, la coque est différenciée en une sorte de couvercle articulé, susceptible de s'ouvrir lorsque l'embryon quittera son enveloppe.



TEXT-FIG. 2.

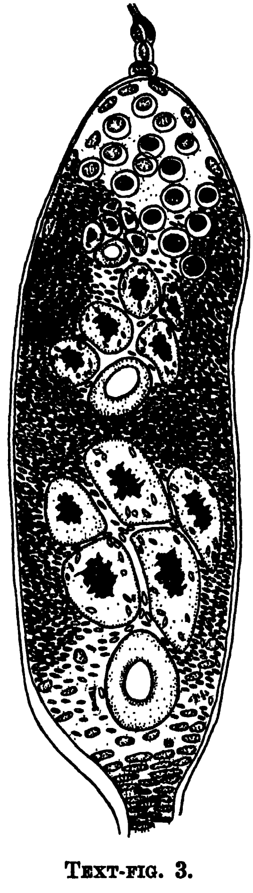
Œuf mûr de Goniodes stylifer. Carmin acétique. Gross.: 105.

La fig. B (fig. 1 du texte) représente un ovaire d'une femelle à sa deuxième mue (III). Il n'y a pas encore de dégénérescence dans la chambre terminale. Deux ou trois groupes de cellules nourricières et d'ovocytes sont differenciés dans chacun des tubes. Un de ceux-ci est dessiné à une plus forte échelle dans la fig. 3 du texte.

Si, dans une préparation au carmin acétique, on ne peut étudier les détails du noyau, on obtient par contre une bonne notion de l'anatomie et de la topographie générale. D'autre part, on est certain d'avoir le nombre total des éléments cellulaires—ce qui peut être d'une grande utilité—et non pas seulement une tranche de tissu plus ou moins bien orientée.

On distingue cinq catégories d'éléments.

(1) Les noyaux folliculaires un peu allongés placés à l'extérieur de la chambre, d'aspect assez semblable à celui des noyaux du filament terminal.



TEXT-FIG. 3.

Tube ovarien d'un ovaire de Goniodes (stade III jeune) à un plus fort grossissement. Carmin acétique. Gross.: 780.

(2) Les ovogonies à noyau croûtelleux.

(3) Les ovocytes dont la chromatine est filamenteuse.

(4) Les cellules nourricières au nombre de cinq.

(5) Le futur œuf.

Les bactéries pullulent dans la zone moyenne du tube.

Les cellules folliculaires vont se diviser activement pour former le revêtement de la première chambre à œuf.

La fig. c (fig. 1 du texte) montre un ovaire à quatre tubes ovariens d'une jeune femelle à sa première mue (II).

Il y a encore peu d'éléments germinaux et les ovogonies sont dans une période de multiplication. Les métaphases permettent la numération de vingt-quatre chromosomes, représentant le nombre diploïde.

Les ovocytes en voie d'accroissement ne se sont pas encore différenciés en cellules nourricières ou germinales.

Quant aux bactéries, elles sont déjà fort nombreuses.

Note sur les Bactéries.

Chez les Pediculides et les Mallophages, l'infestation de l'œuf se fait par l'intermédiaire des cellules à bactéries ou mycetocytes. Les bactéries sortent à travers l'hypoderme de l'insecte juste avant la dernière mue, tombent dans l'espace constitué par l'enveloppe dont l'animal se dépouille et son corps, passent par l'orifice génital et s'accumulent dans une ampoule du tractus génital, dans laquelle viennent déboucher les tubes ovariens. C'est à ce niveau que les œufs sont pénétrés par les bactéries, bien avant que le micropyle soit constitué.

Dans d'autres cas, il y a infestation directe de l'ovocyte. Les bactéries passent alors des mycetocytes dans l'oviducte à travers la paroi du conduit ovarien.

Chez le Goniodes stylifer, le processus semble être bien différent et je me propose de l'étudier de plus près. On retrouve les mycetocytes ou cellules à bactéries dès le stade I, tout au début de la vie de ce Mallophage. Déjà à ce moment peut-être, en tous cas dès la première mue, les tout jeunes ovaires sont infestés par les éléments bactéroïdiens qui s'insinuent entre les cellules germinales. Très rapidement, les cinq cellules différenciées en éléments nourriciers qui coiffent l'ovocyte sont pénétrées par des bactéries qui sont alors logées au sein du protoplasme dans de petites vacuoles claires. L'ovocyte lui-même est, à ce moment, absolument vierge de symbiontes.

Au cours de la résorption des cellules nourricières au profit du futur œuf, il se pourrait qu'il y ait passage de bactéries des éléments nourriciers à l'ovocyte.

Il n'y a pas d'ampoule ovarienne typique chez le Goniodes stylifer et je n'ai jamais observé de bactéries dans la région inférieure de l'oviducte.

L'intérêt réside donc dans l'existence,[1] à un stade très précoce, de nombreuses bactéries dans la chambre terminale des tubes ovariens, puis dans le passage de bactéries dans les cellules nourricières de l'œuf. Quant au mode d'infestation de l'ovocyte, il est difficile de le mettre en évidence car les bactéries se colorent très faiblement par les méthodes usuelles.

Évolution des éléments germinaux jeunes.

Maintenant que nous avons une première idée des éléments germinaux femelles du Goniodes, examinons de plus près leur aspect cytologique.[2]

Dans les préparations d'ovaires les plus jeunes que j'ai pu obtenir (stade I), à part les noyaux du filament terminal et ceux de l'oviducte, on peut toujours séparer de prime abord deux catégories de vésicule nucléaire noyée dans un protoplasme commun.

Certains noyaux sont clairs, renfermant un nucléole mais peu de chromatine; ce sont les éléments folliculaires.

D'autres noyaux au contraire présentent des aspects quiescents avec chromatine croûtelleuse, des prophases et des divisions, qui sont autant de figures d'une même classe d'éléments, les éléments germinaux.

A ce stade très jeune (figs. 40–2, 43–5, Pl. 25) les bactéries n'existent pas encore dans l'ovaire, mais elles ne vont pas tarder à l'envahir.

Dans des ovaires plus évolués de femelle à leur deuxième et troisième mue, il est difficile, à première vue, de se faire une idée exacte de la sériation des éléments. Ceux-ci ne se présentent pas sous un aspect aussi simple que ceux décrits par Ries, et je ne pense pas que Goniodes stylifer soit un cas particulier chez les Mallophages et Pédiculides, mais bien au contraire qu'il est l'expression d'un type général.

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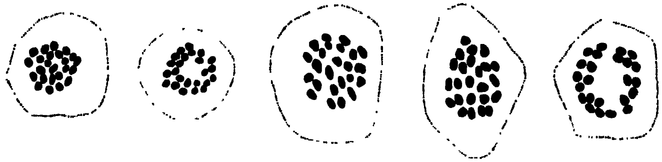
[1] Cette existence ne peut s'expliquer, semble-t-il, que par émigration de bactéries des mycetocytes aux chambres terminales à travers les parois des ovaires.

[2] Il est évident que cette étude ne peut se faire que sur coupes.

Les ovogonies ne présentent pas de membrane cellulaire et les noyaux semblent plongés dans un protoplasme commun.[1] L'indépendance des cellules ne se manifeste que plus tard à la fin de l'évolution goniale ou au début de la croissance des ovocytes.

Les noyaux des ovogonies ont un gros nucléole. La chromatine au repos est répartie en grumeaux et en filaments (fig. 1, Pl. 23).



TEXT-FIG. 4.

Plaques équatoriales de cinèses goniales femelles, présentant vingt-quatre chromosomes. Les deux figures de gauche, d'après des préparations aux liquides de Carothers et de Flemming; les trois figures de droite, d'après des préparations au carmin acétique. Gross.: 2,500.

La prophase, d'aspect bien typique (fig. 1, Pl. 23), amène la mise en plaque équatoriale de ses éléments chromatiques (fig. 2, Pl. 23). On peut compter vingt-quatre chromosomes de dimensions presque égales et qui suivent tous un processus de division normal (fig. 4 du texte).

Lors de l'accroissement des ovocytes jusqu'au stade leptotène, la chromatine se résout graduellement en filaments plus nets — répartis régulièrement dans le noyau — sur lesquels tranchent un ou deux nucléoles (fig. 3, Pl. 23).

La contraction synaptique s'opère alors. Au début de son évolution, les anses chromatiques semblent être encore diploïdes (fig. 4, Pl. 23). La centrotaxie est marquée. Puis les éléments subissent l'appariement synaptique ou pseudoréduction (fig. 5

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[1] Pourtant, dans les préparations au carmin acétique, les cellules goniales ont bien l'air d'être indépendantes.

Pl. 23) suivi bientôt de la décontraction, phénomène précurseur de la pachyténie (fig. 6, Pl. 23).

Ries admet que ces cellules pachytènes, qu'il considère comme des éléments plus jeunes que les stades amphitènes ou synaptiques,[1] sont toujours au nombre de six, représentant ainsi le futur œuf et ses cinq cellules nourricières.

Dans mes préparations de Goniodes, j'ai pu compter le plus souvent huit à douze cellules au stade pachytène. On peut se demander, vu la fréquence de noyaux picnotiques dans les ovaires âgés, si un processus de dégénérescence ne vient pas limiter le nombre de ces éléments. Il se pourrait aussi que l'évolution des cellules germinales ne soit pas parfaitement synchrone et que les huit à douze noyaux pachytènes représentent en fait deux groupes de six, dont le dernier ne serait pas toujours constitué entièrement.

C'est à partir de ce stade que l'on peut suivre la double évolution de ces ovocytes en cellules nourricières et en futur œuf. On remarque de prime abord la taille réduite des éléments d'un groupe déjà différencié par rapport à celle des cellules en pachyténie. Il doit y avoir des échanges osmotiques particuliers pendant cette période, ramenant à des dimensions normales les énormes noyaux pachytènes.

La fig. 7 (Pl. 23) semble être l'image de ce phénomène probablement rapide, qui entraîne un raccourcissement des filaments chromatiques. On ne peut confondre ce stade, qui se trouve en avant[2] des autres éléments, avec la synapsis qui, elle, se passe plus en arrière dans l'ovariole.

Peu après, les six cellules différenciées forment un groupe parfaitement distinct. La chromatine du futur œuf conserve sa structure filamenteuse, tandis que celle des éléments nourriciers commence à se fractionner en grumeaux et qu'un gros nucléole, de forme irrégulière, se maintient au sein du noyau (figs. 8, 9, 10, Pl. 23).

Les figures demi-schématiques A et B (fig. 5 du texte) montrent la position des divers stades d'évolution des éléments germinaux et nourriciers.

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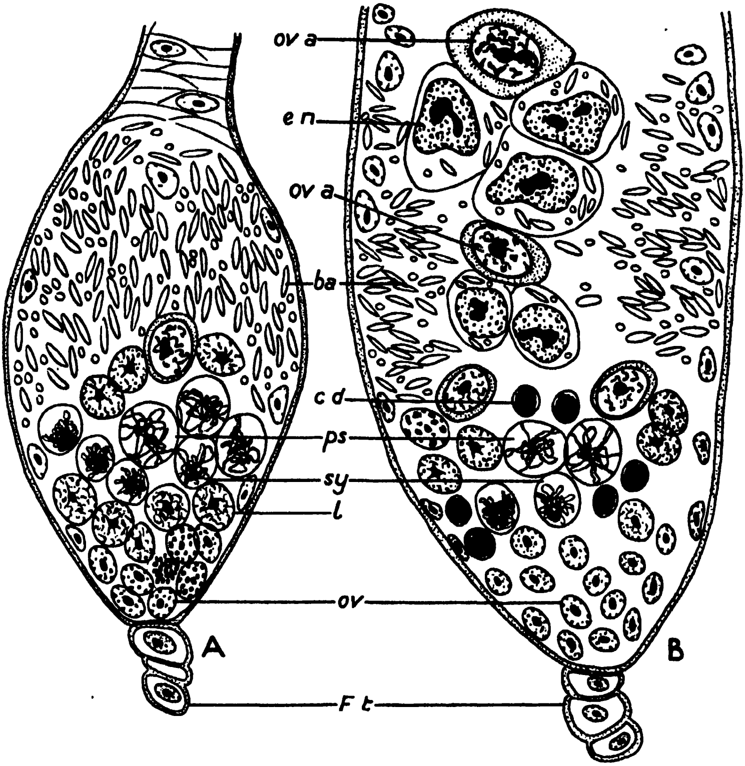
[1] Cette interprétation, certainement erronée, provient du fait que les ovocytes sont souvent mélangés et n'offrent pas toujours une sériation parfaite.

[2] C'est-à-dire plus près de l'oviducte.



TEXT-FIG. 5.

A. Coupe d'un tube ovarien d'un individu à sa première mue (stade II). Demi-schématique. B. Coupe d'un tube ovarien de femelle au stade III. *Ft.*, filament terminal; *ov.*, ovogonies; *l.*, leptoténie; *sy.*, synapsis; *ps.*, pachyténie; *en.*, éléments nourriciers; *ov.a.*, ovocytes avancés; *c.d.*, cellules en dégénérescence. *ba.*, bactéries. Préparations au liquide de Flemming; coloration à l'hématoxyline. Gross.: 1,500.

La fig. A représente la coupe d'un ovariole de jeune femelle à sa première mue (stade II).

La fig. B est l'image d'un tube plus âgé correspondant à un individu au stade III.

Évolution de l'ovocyte dans la chambre à œuf.

Suivons maintenant rapidement l'évolution de l'ovocyte qui précède ses cinq cellules nourricières — lesquelles recouvrent son extrémité postérieure comme d'une calotte — et qui s'engage plus bas dans l'ovariole en se séparant ainsi du groupe suivant.

Bien que Ries ait étudié en détail le développement de l'œuf des Mallophages et Pediculides, je reprendrai brièvement cette description chez le Goniodes stylifer.

Les cellules folliculaires se multiplient activement par division indirecte, et viennent former une assise cellulaire autour de l'ovocyte. Celui-ci croît aux dépens des cellules nourricières, élabore du deutoplasme en abondance, et, lorsque les restes des éléments nourriciers en dégénérescence ont disparu, remplit toute la chambre à œuf.

Après leur première phase de multiplication intense, les cellules folliculaires de l'enveloppe ne présentent plus de mitoses, mais leurs noyaux allongés, et même souvent étranglés, mettent hors de doute l'existence de divisions amitotiques.

Les noyaux des cellules nourricières, de forme souvent irrégulière, contiennent, au début de leur évolution, des granulations chromatiques nombreuses, colorées par la méthode à la fuchsine sulfureuse, et provenant de la désintégration des filaments préexistants (fig. 10, Pl. 23). Quelques nucléoles, par contre, prennent le vert lumière. Très rapidement, ces granulations ne se colorent plus au Feulgen. Il se passe alors des transformations importantes dans les éléments nourriciers que Ries a étudiés en détail et qu'il est inutile de développer ici (figs. 46–8, Pl. 25).

Évolution du noyau du futur œuf.

Dès que le futur œuf, suivi de ses cinq cellules nourricières, commence à quitter la chambre terminale, et s'isole des autres éléments germinaux plus jeunes, son noyau, dans la double coloration Feulgen — vert lumière, tend à se colorer par le dernier agent. Cette transformation chimique du noyau se passe même avant celle, déjà citée, de la chromatine des cellules nourricières[1] (fig. 11, Pl. 23).

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[1] Ceci concorde avec ce que dit Jean Brachet. Cet auteur retrouve, dans l'ovogenèse des Lamellibranches, des Gastéropodes, des Amphibiens, des Reptiles et des Mammifères, un stade où la réaction de Feulgen est négative

On devine encore longtemps des filaments et un nucléole (fig. 12, Pl. 23). Puis la membrane nucléaire se détend, se frippe, prend un aspect godroné (fig. 13, Pl. 23). A ce moment, le noyau est d'une teinte presque semblable à l'aire cytoplasmique avoisinante. C'est à ce stade que Ries décrit la formation des chromidies. Un réseau plus foncé, reste de la substance chromatique, est amassé au centre de la vésicule nucléaire (fig. 14, Pl. 23).

Plus tard encore, alors que l'ovocyte a atteint des proportions considérables aux dépens des cellules nourricières, l'aire nucléaire est à peine visible. Une zône un peu plus dense représente tout ce qui reste du noyau dont la membrane a disparu (fig. 15, Pl. 23).

C'est à ce stade ultime que succède l'évolution du micronoyau. On observe alors une vésicule qui tend très nettement à se colorer par la fuchsine sulfureuse (fig. 16, Pl. 23), qui se condense de plus en plus en augmentant de colorabilité et qui offre bientôt l'image d'une sorte de peloton rouge violacé au milieu d'une aire cytoplasmique jaunâtre (fig. 17, Pl. 23).

Le dernier stade qu'il m'a été permis d'étudier, est la prophase de la première division de maturation (fig. 18, Pl. 23). Le micronoyau consiste alors en une vésicule nucléaire très réduite dans laquelle sont répartis les éléments chromosomiques condensés, qui attendront la maturité de l'œuf et probablement la pénétration du spermatozoïde pour entrer en métaphase.

La spermatogenèse de Goniodes stylifer.

Région goniale.

Ce n'est que dans des préparations d'individus à leur première mue (stade II) que j'ai pu déceler pour la première fois les testicules, mais il semble que ceux-ci doivent être différenciés bien plus tôt, et qu'ils n'échappent à l'observateur que par leur exiguïté. On trouve déjà à ce moment des spermatocytes en croissance, mais la majeure partie de la glande est constituée par les spermatogonies (fig. 23, Pl. 24) dont les plus petites surtout,

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au niveau des chromosomes, c'est la période intermédiaire d'accroissement des ovocytes.

qui représentent les éléments goniaux les plus âgés, sont nettement groupées en rosettes (fig. 20, Pl. 24).

Les cystes, parfaitement distincts les uns des autres, contiennent, au fur et à mesure de leur évolution, un nombre toujours plus grand de cellules. On peut compter soixante à soixante-dix noyaux dans les acini goniaux les plus évolués et ce chiffre maximum permet d'évaluer le nombre des divisions spermatogoniales à six.

Dans la région apicale du testicule qui, elle, n'est pas délimitée en cyste, mais qui présente des noyaux sans contours cellulaires marqués, on distingue plusieurs catégories d'éléments (fig. 19, Pl. 24).

A côté des spermatogonies qui diffèrent suivant leur âge et leur état par leurs dimensions et l'aspect de leur vésicule nucléaire, on trouve des noyaux — qui, à l'apex, sont encore relativement grands, mais qui diminuent de taille au cours de leur évolution, — provenant des éléments épithéliaux de la paroi des cystes (fig. 20, Pl. 24).

D'autres noyaux par contre, remarquables par leur taille plus du double de celle des noyaux goniaux, paraissent être des éléments germinaux isolés et géants (fig. 35, Pl. 24). Si cette interprétation est exacte, le fait pourrait être rapproché de l'existence de cellules énormes tétraploïdes ou même octoploïdes que l'on trouve fréquemment dans les testicules d'insectes et de vertébrés, cellules ayant peut-être la même origine que les spermatogonies, mais qui ont évolué anormalement.

On pourrait admettre aussi, avec Doncaster et Cannon, que ces cellules, qui présentent le nombre de chromosome diploïde (figs. 37–9, Pl. 24) avec une netteté absolue (24) ne sont que des éléments somatiques noyés dans la glande germinale.

Dans la région inférieure du testicule où se développent les spermatogonies les plus âgées et les spermatocytes, on ne rencontre plus ces cellules géantes qui sont donc localisées dans la zone encore peu différenciée de la glande.

Nous avons vu que les divisions successives des spermatogonies devaient être au nombre de six et que les derniers cystes, avant l'évolution cytaire, contenaient de soixante à soixante-dix cellules.

Les métaphases goniales (fig. 22, Pl. 24) montrent toujours douze chromosomes, correspondant au nombre haploïde. Les prophases (fig. 21, Pl. 24) présentent parfois des éléments chromatiques allongés (fig. 39, Pl. 24), mais ce n'est pas une raison suffisante, me semble-t-il, pour conclure a priori à une prosyndèse (Doncaster), explication évidemment facile d'une pseudohaploïdie goniale.

Évolution des spermatocytes.

Les spermatocytes subissent une longue évolution avant d'arriver à la division hétéropolaire (figs. 24 et 25, Pl. 24).

Ce qui les distingue de prime abord des spermatogonies, à part leur taille plus grande, ce sont deux corps cytoplasmiques qui prennent rapidement naissance et qui serviront à la formation du spermatozoïde.

Le premier est appelé 'mitosome' par Doncaster et Cannon. Il représente une agglomération d'éléments mitochondriens, futur 'nebenkern'.

Le second est nommé 'acroblaste' et deviendra l'acrosome. C'est à son niveau qu'apparaissent les centrosomes en V de la cinèse hétéropolaire.

Le noyau du spermatocyte reste au repos pendant une longue période, puis devient plus nettement filamenteux (fig. 26, Pl. 24). A ce moment a lieu une contraction de la chromatine (fig. 27, Pl. 24), qui ne peut être l'homologue de la vraie synapsis — qui, elle, implique l'idée de parasyndèse, de pseudoréduction par accollement des deux filaments d'une même paire — mais qui, correspond à la seconde contraction de Wilson, se plaçant juste avant la diakinèse.

Les anses chromatiques sont pelotonnées autour d'un nucléole, mais il n'y a pas de centrotaxie et le noyau ne diminue pas de taille. Tout porte à croire que cet état contracté se passe à la fin d'une longue évolution du spermatocyte — la vraie synapsis devrait se passer au début. Topographiquement, les cystes présentant des cellules dans cet état, sont voisins de ceux qui contiennent les prophases ou métaphases de la cinèse hétéropolaire. Les éléments cytoplasmiques, mitosomes, acroblastes, centrosomes, sont tous bien formés, ce qui plaide en faveur de notre hypothèse. Il est intéressant de noter que, chez Pediculus humanus, cette contraction n'existe pas. La diakinèse suit immédiatement les stades de repos du noyau. La spermatogenèse de Goniodes est identique, dans ses points principaux, à celle de Pediculus. Le stade de contraction chromatique n'est point donc essentiel, ce qui semble exclure l'idée qu'il représenterait une vraie synapsis.

Par un rapide processus de condensation (fig. 28, Pl. 24) et sans passer par la pachyténie et diploténie, le noyau se trouve au stade diakinétique (fig. 29, Pl. 24). On peut compter douze éléments chromatiques fissurés longitudinalement, qui sont les dyades, et leur fissuration semble correspondre à la division équationnelle. (Voir discussion.)

A ce moment, la membrane nucléaire disparaît, les centrosomes, jusqu'alors appliqués contre le noyau, s'éloignent, et les dyades, tout en prenant l'aspect globuleux, se mettent au fuseau (fig. 30, Pl. 24).

La métaphase présente des chromosomes très compacts (fig. 31, Pl. 24). Il est difficile d'en compter douze, mais un peu plus tard — lorsqu'une sorte de digitation protoplasmique se forme à l'un des pôles de la cellule et que le centrosome correspondant s'y engage, entraînant ainsi l'allongement du fuseau, — on arrive souvent à numérer les douze chromosomes prêts à se diviser équationnellement (fig. 32, Pl. 24).

L'anaphase, rarement observable, est du reste tellement serrée qu'on ne peut suivre l'évolution des chromosomes. A la télophase, le processus digité — si semblable au globule polaire des spermatocytes d'Abeille — s'étrangle de plus en plus, emprisonnant dans une portion cytoplasmique très réduite, la moitié de la chromatine (fig. 33, Pl. 24). Ainsi se termine cette unique division du spermatocyte, division hétéropolaire, qui amène la formation d'une seule spermatide.

Dans les cystes contenant ces derniers éléments en évolution, on observe encore longtemps les restes en dégénérescence de ces globules polaires (fig. 34, Pl. 24).

Il y a donc formation de spermatozoïdes en nombre égal à celui des spermatogonies. C'est ce qu'il est facile de prouver par des numérations. En effet, les faisceaux des spermatozoïdes d'un même cyste comportent tous de soixante à soixante-dix éléments, ce qui correspond au nombre de spermatogonies dans les cystes les plus évolués de la région goniale.

Discussion et Conclusions.

Doncaster et Cannon, observant des chromosomes couplés en nombre haploïde dans les prophases des jeunes spermatogonies, admettent un phénomène de prosyndèse analogue à celui qui se passe chez les Diptères. Cette idée est confirmée, d'après eux, par le fait de l'existence de chromosomes doubles dans la prophase des spermatocytes. Ainsi ces auteurs sont amenés à considérer l'unique division hétéropolaire des spermatocytes comme la cinèse hétérotypique, — qui ne serait donc pas suivie d'une division homéotypique dissociant les moitiés d'éléments univalents fissurés longitudinalement, ce qui les rend très perplexes au sujet de la valeur de leur hypothèse.

Avant de discuter cette interprétation, voyons tout d'abord les faits que mettent en évidence l'étude de Doncaster et Cannon sur Pediculus, et la mienne sur Goniodes.

La femelle possède un nombre diploïde de chromosomes dans ses cellules somatiques.

Les métaphases ovogoniales présentent ce même nombre.

Il existe dans l'ovogenèse des stades leptotènes, amphitènes et pachytènes.

D'après l'étude de Ries, il semble que les divisions réductionnelles — au moins la première — soient normales.

La femelle présente donc un cycle chromosomique tout à fait normal.

Le mâle a dans ses cellules somatiques le nombre diploïde de chromosomes.

Les cellules géantes[1] du testicule présentent le nombre diploïde de chromosomes, mais ces éléments ne peuvent être considérés comme jouant un rôle dans la spermatogenèse, qu'on les nomme cellules somatiques ou cellules anormales.

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[1] Ces cellules semblent géantes lorsqu'elles sont au milieu de spermatogonies évoluées, deux ou trois fois plus petites, mais cette différence est bien moins marquée dans la région apicale du testicule, où les éléments germinaux sont encore d'une taille relativement grande.

Les spermatogonies montrent des prophases qui ont des chromosomes en nombre haploïde. Mais ces prophases, surtout dans les éléments très jeunes du testicule au début de son évolution, suggèrent l'idée d'un accouplement des éléments homologues en nombre diploïde.

Les métaphases sont d'une netteté absolue montrant le nombre réduit de chromosomes globuleux.

Les spermatocytes ont une longue phase de repos, suivie d'une contraction chromatique (cas du Goniodes) ('second contraction' de Wilson) amenant rapidement la diakinèse. Ce stade peut manquer. (Cas du Pediculus). La diakinèse suit alors immédiatement le stade du noyau au repos.

Il n'y a pas de vraie synapsis, ni de pachyténie.

La diakinèse présente des éléments fissurés longitudinalement comme de typiques dyades — ou des chromosomes accouplés (Doncaster).

Il n'y a qu'une division des spermatocytes, qui, comme nous le verrons tout à l'heure, peut être équationnelle, dissociant les deux moitiés des dyades.

Cette cinèse est hétéropolaire quant au cytoplasme et n'amène donc la formation que d'une spermatide.

On peut faire deux hypothèses pour expliquer la spermatogenèse toute particulière du Poux.

Première hypothèse.

L'œuf est fécondé, le nombre diploïde est rétabli. Il se passe un phénomène de prosyndèse à un stade très jeune de l'évolution germinale, phénomène remplaçant la pseudoréduction synaptique qui est la règle chez tout organisme diploïde. Ce serait en sorte un état pachytène très précoce.

L'unique division des spermatocytes représenterait alors la cinèse hétérotypique, dissociant les deux éléments des paires chromosomiques.

Par le fait même qu'il n'existe pas de phénomènes synaptiques et pas de formations tétradiques, cas tout à fait anormal, il n'y a pas nécessité d'une division homéotypique subséquente. On peut fort bien admettre l'existence d'une cinèse réductionnelle non suivie d'une cinèse équationnelle dans un cas semblable, d'autant plus que les processus cytologiques qui, dans la règle, précèdent les deux divisions, n'existent pas ici.

Deuxième hypothèse.

L'œuf n'est pas fécondé, il se développe parthénogénétiquement en donnant un mâle.

Il y a rétablissement de l'état diploïde, soit par fusion du noyau du second globule polaire avec celui de l'œuf (cas du Lecanium, Thomsen), soit par simple dédoublement des chromosomes à un stade blastomérique précoce (cas de l'Abeille, P. Hertwig et Rana, Parmenter).

L'embryon est alors diploïde et homozygote.

Dans le testicule, il y a retour à un état pseudo-haploïde aux tout premiers stades goniaux par un simple processus d'accouplement des éléments d'une même paire.

Il est dès lors évident que les phénomènes pseudoréductionnels, tels que la synapsis et la pachyténie, précurseurs d'une division hétérotypique, sont inutiles, puisque les deux éléments d'une même paire chromosomique sont de même origine maternelle.

Il ne subsiste alors que la seconde division de maturation qui est homéotypique.[1]

Un point intéressant qui ressort de ces observations si difficiles à interpréter, c'est le double fait de l'absence de stades préméiotiques ainsi que d'une des deux divisions de maturation. On observe la même chose dans l'ovogenèse des organismes parthénogénétiques. Il y a, comme le remarque Vandel, une série d'intermédiaires entre la parthénogenèse facultative et la parthénogenèse constante, qui nous montre l'oblitération progressive des phénomènes réductionnels et synaptiques, aboutissant au cycle entièrement diploïde. A l'exception du cas des Diptères mâles, où les phases préméiotiques font défaut, mais où les deux divisions de réduction sont présentes, on peut prétendre que l'absence des stades synaptiques va de paire avec l'existence d'une seule division de maturation. La spermatogenèse du Poux est un exemple de la liaison des deux phénomènes.

---

[1] En employant ici les mots 'hétérotypique' et 'homéotypique', je me place au point de vue du généticien.

Nous voyons donc que l'étude cytologique a elle seule, ne suffit pas pour résoudre le problème. Ce n'est que par une méthode d'élevage appropriée qu'on atteindra ce résultat. Malheureusement, ce travail offre de grandes difficultés techniques. Les Mallophages meurent rapidement en captivité. Le Pediculus humanus, qui semble être le matériel le meilleur, demande cependant plusieurs nourrissages par jour sur l'homme même.

Les expériences d'élevage de Hindle et de Doncaster et Cannon n'amènent pas d'éclaircissement, les premières parce qu'elles ont été effectuées sur une base trop réduite, les secondes parce que leurs résultats n'offrent aucune homogénéité.

Il y a là tout un champ d'étude susceptible de faire apparaître la vérité. Sans de telles recherches, la seule observation cytologique est vaine.

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EXPLICATION DES PLANCHES 23 à 25.

PLANCHE 23. L'OVOGENÈSE.

Fig. 1.—Ovogonie et prophase goniale. Flemming, Hematoxyline.

Fig. 2.—Plaque équatoriale goniale (vingt-quatre chromosomes). Flemming, Hemat.

Fig. 3.—Évolution de l'ovogonie en ovocyte. Flemming, Hemat.

Fig. 4.—Leptoténie et synapsis. Flemming, Hemat.

Fig. 5.—Contraction et décontraction synaptiques. Carothers, Hemat.

Fig. 6.—Pachyténie. Carothers, Hemat.

Fig. 7.—Évolution du noyau pachytène en élément purement germinal (noyau du futur œuf). Flemming, Hemat.

Fig. 8.—Cellule nourricière et cellule œuf. Flemming, Hemat.

Fig. 9.—Trois cellules nourricières, dont la chromatine est en train de se fragmenter, et cellule œuf. Flemming, Hemat.

Fig. 10.—Élément nourricier et cellule œuf, plus évolués. Flemming, Hemat.

Ces dix figures ont été dessinées avec la combinaison optique suivante: oculaire 25×, objectif 1/16, ce qui donne un grossissement de 5,400, et après réduction de 2/5, un grossissement de 3,240.

Fig. 11.—Cellule œuf, environ au stade représenté à la fig. 10.

Fig. 12.—Début du stade intermédiaire de grossissement de l'ovocyte où la réaction de Feulgen est presque négative.

Fig. 13.—La membrane nucléaire se détend.

Fig. 14.—La chromatine pâle se concentre au milieu de la vésicule nucléaire godronée.

Fig. 15.—Un amas de substances nucléaires est à peine visible au milieu de l'aire nucléaire.

Fig. 16.—Réapparition du noyau.

Fig. 17.—Peloton chromatique très positif à la réaction de Feulgen.

Fig. 18.—Prophase de la première division de maturation.

Les figs. 11 à 18 ont été dessinées avec la combinaison optique suivante: oculaire 12×, objectif 1/16, ce qui donne un grossissement de 2,500, et après réduction de 2/5, un grossissement de 1,500. Fixateur, Flemming; coloration, Feulgen.

PLANCHE 24. LA SPERMATOGENÈSE.

Fig. 19.—Filament terminal, spermatogonies au repos et en divisions (douze chromosomes).

Fig. 20.—Zone goniale du testicule, mais plus évoluée. Éléments pitéhéliaux, éléments germinaux groupés en rosettes, télophases goniales.

Fig. 21.—Prophase goniale (douze chromosomes et un nucléole).

Fig. 22.—Métaphase goniale (douze chromosomes).

Fig. 23.—Spermatogonies.

Fig. 24.—Spermatocytes. L'acroblaste prend naissance au sein du cytoplasme.

Fig. 25.—Spermatocytes plus évolués, chromatine en réseau. Au niveau de l'acroblaste, deux corps qui donneront peut-être les centrosomes. Les mitosomes sont déjà formés.

Fig. 26.—Chromatine plus ou moins filamenteuse.

Fig. 27.—Contraction chromatique pseudosynaptique. Les stades 26, 27, et 28 manquent complètement chez Pediculus.

Fig. 28.—Formation des dyades.

Fig. 29.—Prophase avec dyades typiques en nombre haploïde (12).

Fig. 30.—Mise en fuseau.

Fig. 31.—Métaphases.

Fig. 32.—Formation de la digitation protoplasmique et étirement du fuseau.

Fig. 33.—Télophase. La digitation s'étrangle en détachant un véritable globule polaire.

Fig. 34.—Spermatides et globules polaires en voie de dégénérescence.

Fig. 35.—Spermatogonies et cellule diploïde.

Fig. 36.—Prophase d'une cellule diploïde.

Fig. 37.—Métaphase (vingt-quatre chromosomes) d'une cellule diploïde.

Figs. 38 et 39.—Deux plaques équatoriales de cellule diploïde au milieu d'éléments germinaux goniaux.

Toutes les figures de la planche II ont été faites avec la combinaison optique suivante: oculaire 25×, objectif 1/16, ce qui donne un grossissement de 5,400 et après réduction de 2/5, un grossissement de 3,240. Fixateur, Flemming; coloration, hematoxyline.

PLANCHE 25.

Figs. 40, 41, and 42.—Jeune tube ovarien de femelle au stade II très jeune. Les trois coupes constituent le tube entier.

Figs. 43, 44, and 45.—Jeune tube ovarien de femelle au stade I. Les trois coupes représentent le même tube dans sa totalité. Cinèses goniales, ovogonies, éléments somatiques du filament terminal et de l'oviducte.

Figs. 46, 47, and 48.—Évolution des éléments nourriciers et de l'ovocyte dans des cystes ovariens jeunes. La chromatine des cellules nourricières se colore encore au Feulgen (fig. 46) alors que le noyau de l'ovocyte prend le vert lumière et que sa membrane commence à se plisser.

Les figs. 40 à 45 ont été dessinées à un grossissement de 2,500. Après réduction de 1/3, le grossissement est de 1,550. Fixateur, Flemming; coloration, hematoxyline.

Les figs. 46, 47, et 48 ont été dessinées à un grossissement de 220, ce qui donne après réduction de 1/3 un grossissement de 150 environ. Fixateur, Flemming; coloration, Feulgen.

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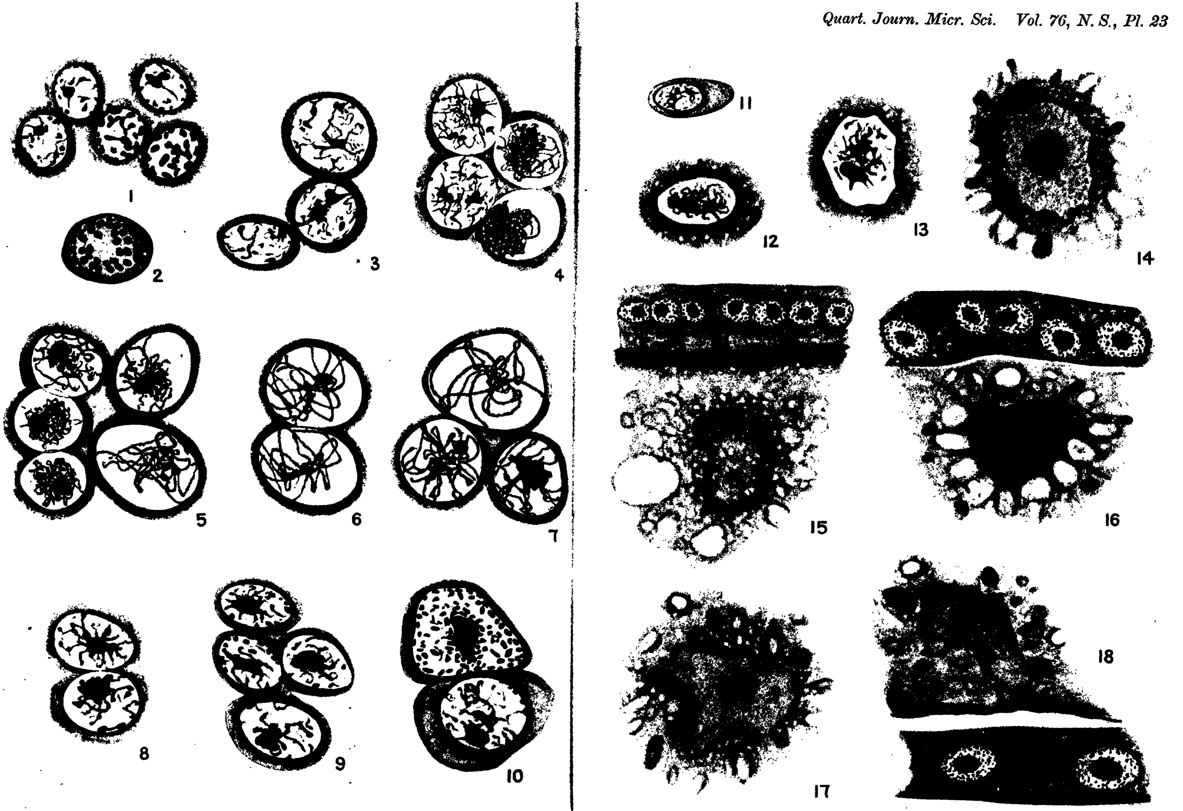

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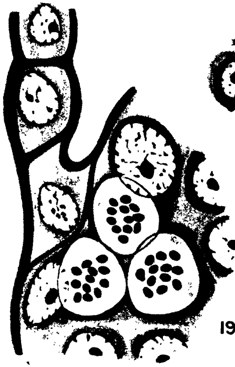

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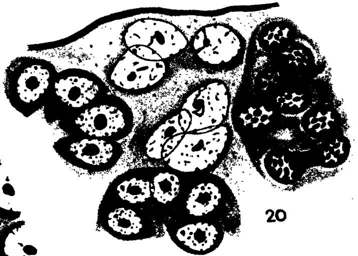

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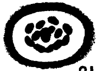

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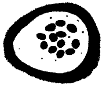

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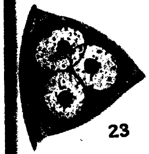

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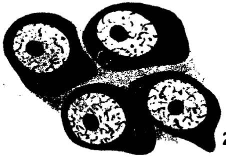

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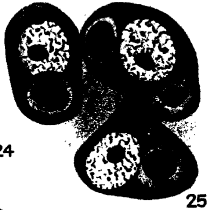

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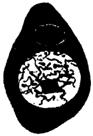

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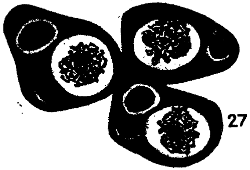

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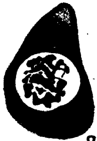

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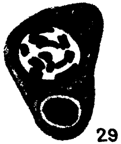

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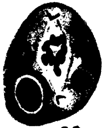

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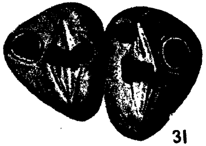

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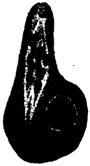

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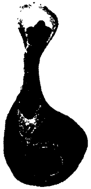

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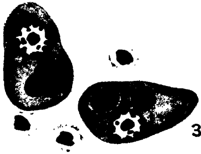

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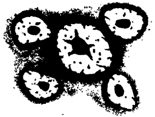

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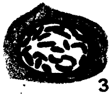

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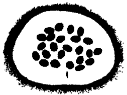

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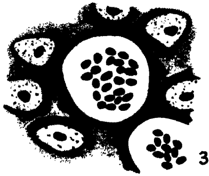

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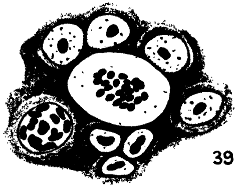

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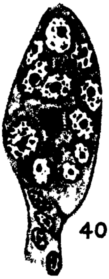
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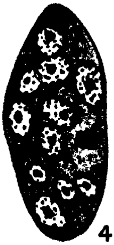
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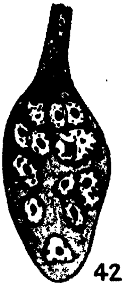
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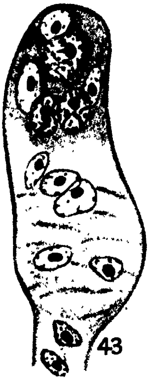
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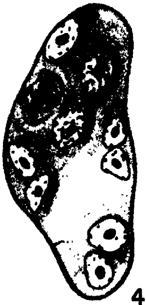
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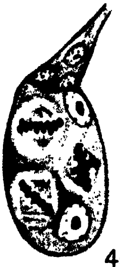
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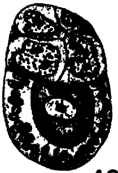
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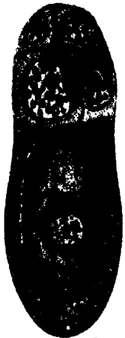
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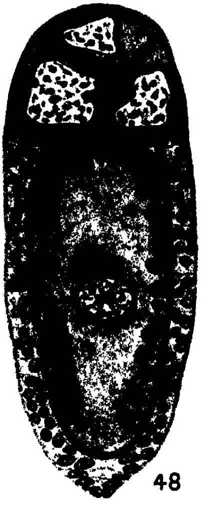
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J. L. Perrot, del.

On the Nature and Functions of the Amoebocytes of Ostrea edulis.

By

Shun-ichi Takatsuki.

(Tokyo University of Literature and Science, Japan.)

With 12 Text-figures.

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INTRODUCTION.

RECENT works by Yonge (1926, 1928) on the behaviour of the amoebocytes of the oyster have called attention to their great importance in the functioning of this animal. The present research was undertaken at the suggestion of Professor C. M. Yonge. A great deal of ground is covered in this paper, so that it has been impossible in the time available to investigate and discuss in detail every problem. It is hoped in future to carry out further investigations.

It is with great pleasure that I take this opportunity to express my indebtedness to Professor C. M. Yonge, for having first introduced me to the subject of these investigations, and for subsequent aid, criticisms, and encouragement throughout the work, and also for the preparation of this manuscript for the press.

I am also greatly indebted to Dr. E. J. Allen, F.R.S., the Director of the Laboratory of Marine Biological Association at Plymouth, for facilities for this research and to him and the other members of the Staff for their kindness and help.

I wish to express my thanks to Mr. A. V. Mitchell for his help in the preparation of this manuscript.

2. MORPHOLOGY OF THE AMOEBOCYTES.

A. Previous Work.

Many investigators have studied the blood-cells of Lamellibranchs. The first description of the blood-cells of Mollusca, so far as I am aware, is by Poli (1791) in Arca glycimeris. In 1848, Wharton gave a comprehensive account of the blood-cells in various groups of invertebrates including the molluscs, Buccinum and Mytilus, and subsequently Lieberkühn (1854), Hessling (1859), Keferstein (1862–6), Flemming (1878), Apathy (1888), Cattaneo (1889), Cuénot (1891), Griesbach (1891), Knoll (1893), De Bruyne (1895), Carazzi (1896), Kollmann (1906), Drew (1910), Goodrich (1919), Canegallo (1924), and others described the morphology of the cells of numerous molluscs. But the conclusions reached, especially with reference to classification and morphological features, were very varied. Wharton divided the blood-cells of Mytilus into three types, and stated that they are transitional stages in development. Cattaneo, Griesbach, and Knoll studied the blood-cells of a large number of Lamellibranchs and divided the cells into two types according to the presence or absence of the granules. De Bruyne gave a lengthy account of these cells in the Lamellibranchs, taking as his types Mytilus, Ostrea, Unio, Anodonta, and he recognized seven varieties of corpuscles.

Cuénot divided the cells into three types according to the stage in development, coarsely and finely granular corpuscles, and a third variety consisting of a nucleus with very little surrounding protoplasm. He considered that these are all derived degenerative changes from the coarsely granular form. Drew described the blood-cells of Cardium, and also recognized three types which agree with Cuénot's classification. Canegallo recognizes three types in the blood-cells of Anodonta: lymphocytes, leucocytes, and granular leucocytes.

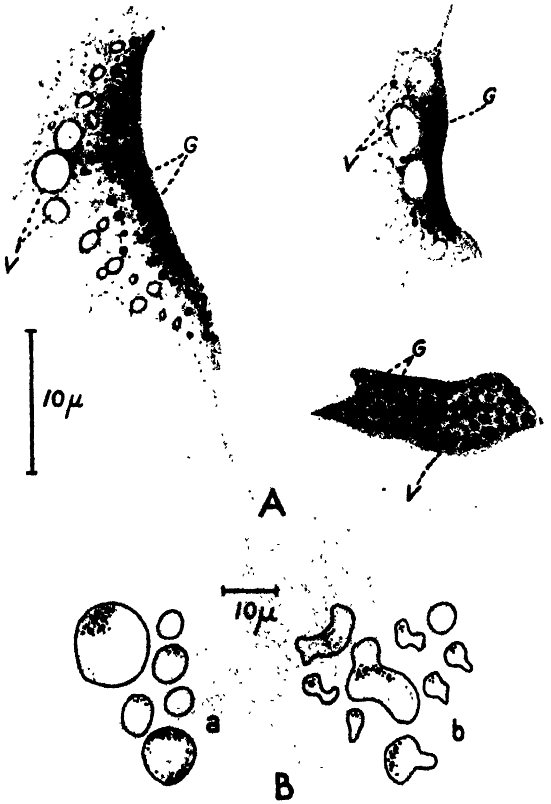
There are various descriptions of the blood-cells of the oyster. Ryder (1882) states that they are about $\frac{1}{300}$th of an inch in diameter, but vary somewhat; but he gives no description of the types of blood-cells. Herdman and Boyce (1899) in describing the blood-cells of the oyster state that 'The blood-cells are normally colourless leucocytes, measuring on an average 10μ in diameter, some, however, are granular, and the granules may be yellow, brown, black, or green in colour'. Carazzi (1896) in his work on the blood-cells of the green oyster states that there is one type, but that some contained many green granules. Griesbach (1891) and Lankester (1885) observed the green granules of amoebocytes of the oyster and their power of amoeboid movement, but they did not distinguish between different types. Orton (1924) mentioned that 'the blood-cells of the oyster are of two main kinds, namely, large granular ones, and large and small clear ones'. He states that the appearance of the large granular form taken fresh from the body is well shown by Herdman and Goodrich, but he gives no description or figures of the clear types.

B. General Description.

The organized elements in the blood of Ostrea edulis consist of two main kinds of amoebocytes. One is the granular (granular leucocytes), the other more hyaline (lymphocytes). Both types can be easily distinguished in fresh unstained preparations of the blood from the heart.

(i) Granular leucocytes (Text-fig. 1 A).

These are relatively large and granular. The cytoplasm contains a number of granules, which, when fresh, are yellow or yellowish green in colour, and vary in size and number. These leucocytes measure 9μ to 13μ in diameter when they are contracted. The nucleus is simple and compact, not lobed or divided, and 3μ to 4μ in diameter. (Carazzi stated that the



TEXT-FIG. 1.

The two kinds of amoebocytes from the heart. Drawn from life. A. Granular amoebocytes in the expanded condition; *G.*, granules; *V.*, vacuoles. B. Hyaline amoebocytes. *a.*, spherical condition; *b.*, the change of form after about twenty minutes.

nucleus of the amoebocytes in the oyster is divided into fragments, but I never observed such a condition.) The leucocytes are amoeboid, varying in size and form, but the rate of progression is slow. The cell puts out delicate thin lamellae round the periphery forming the so-called 'pseudopodia' (see Section F). The fresh granular amoebocytes which are collected from the mantle cavity or gill surface readily unite with one another by means of bristle-like pseudopodia, but the boundaries of the cells can always be identified as the cells do not actually coalesce (Text-fig. 2 D).

(ii) Lymphocytes (Text-fig. 1 B).

These are never entirely hyaline; the cytoplasm sometimes contains a few granules. In fresh unstained preparations of the blood, they can easily be distinguished by their spherical shape and high power of refraction. They are pale in colour and very variable in size, 5μ to 15μ in diameter. Amoeboid movement is less marked in them than in the preceding type; they do not expand to the same extent or produce bristle-like pseudopodia.

In the course of examining these two kinds of amoebocytes, observations were made on fresh preparations in a moist chamber and examined at intervals. The granular leucocytes are actively amoeboid although the rate of progression is slow. The amoebocytes present an indefinite variety of forms when they move. After the lapse of about half an hour many become fully extended, and may then measure three or four times their length in the contracted state. In this condition they are very thin, sometimes transparent, and can be most conveniently observed under a narrow cone of illumination. Some of them link up together to form an open meshwork of chains of cells. Adjacent cells are joined up by cylindrical processes recalling the axons of a bipolar nerve-cell. The expanded amoebocytes show large and small vacuoles in the cytoplasm, and granules can easily be identified in this condition.

On the other hand, the amoeboid movement of the lymphocytes is very simple; they produce lobe-like pseudopodia and change their form, but not very actively.

C. Chromatic Properties of Granules.

There are several descriptions of the natural colour of the granules in the amoebocytes of the oyster; there are no detailed descriptions of their chromatic properties.

The granules of the amoebocytes are usually yellow or yellowish green when fresh, infrequently they are brown; but the black colour recognized by Herdman and Boyce was never seen. In the so-called 'green oyster' the colour is more greenish. The number of the granules varies in individual amoebocytes. Drew and Cuénot recognized two kinds of granules as above mentioned, but the granular contents of the amoebocytes of the oyster cannot be so divided. The following examination was undertaken with the hope of determining the affinity of the granules for dyes when in fresh condition.

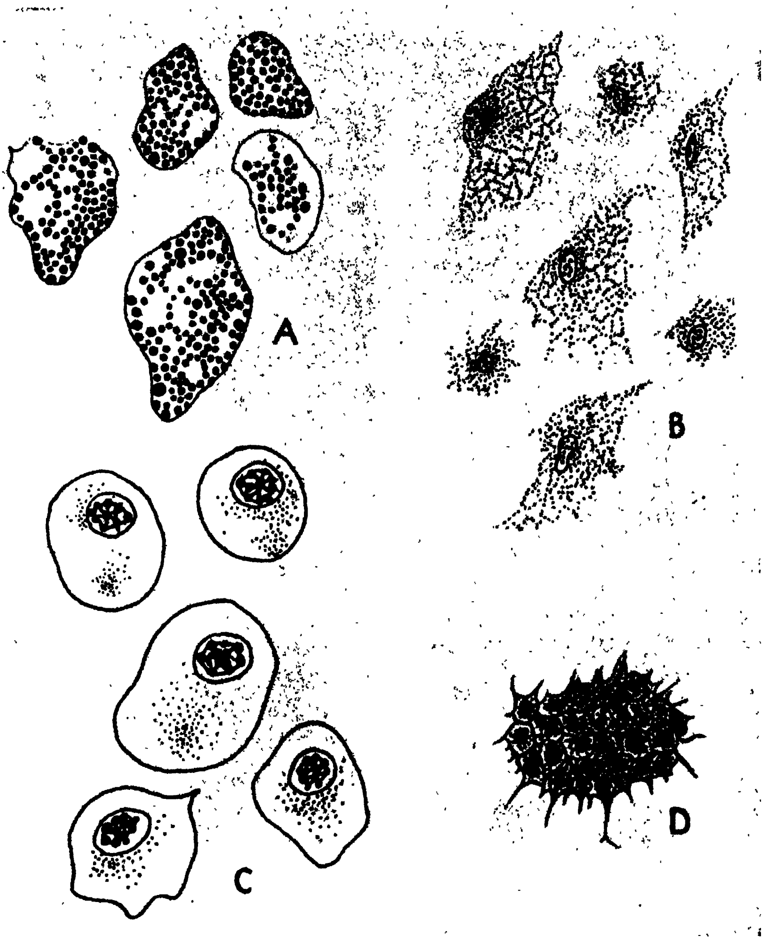
1. Methylene blue, Methyl violet B, and Neutral red were used. The granules are stained by these dyes when fresh, but the dilution of the dyes is a very important factor; certain concentrations stain only the nucleus, but not the granules.

(i) Methylene Blue.

0·1 per cent. methylene blue was first employed. The result was satisfactory for the nucleus, which always stained well, but never more than a small number of granules were stained. The amoebocytes are very sensitive to such a concentration; they change their form and become spherical, and the granules congregate eccentrically. When treated for about half an hour in the moist chamber with a more dilute solution, e.g. 1/10,000 or 1/100,000 strength, the granules stained vividly, but the nucleus only faintly.

(ii) Neutral Red.

This dye has a great affinity for the granules, which become red in dilute solution, and in certain concentrations a brownish red. Solutions of about 1/10,000 and 1/100,000 were used, and good results were always obtained. The stained granules varied in size, but they were never found in the delicate lamella. The



TEXT-FIG. 2.

A. Granules stained with neutral red intra vitam. ×450. B. Fixed and stained expanded amoebocytes in blood-film preparation. ×450. C. Fixed and stained amoebocytes found in vesicular tissue round the stomach. D. Aggregated amoebocytes. Drawn from life. ×250.

results are shown in Text-fig. 2 A. The nucleus never stained with this dye (Text-fig. 2 A).

(iii) Methyl Violet B.

The granules also stained with dilute solutions of this dye in much the same manner as with methylene blue, but not so clearly as with neutral red.

2. Leishman's Stain. The granules are stained reddish purple by this dye, and there are no granular amoebocytes having such fine or coarse granules as those described by Cuénot and Drew. The cytoplasm is not stained clearly, except that the contracted amoebocytes appear a faint bluish purple. The nucleus is stained blue or purple.

3. Giemsa's Stain. As a result of staining with this dye, the granules appear reddish violet, and the nuclei brilliant purple. The granules in the amoebocytes cannot be divided into different types.

When stained with Leishman and Giemsa careful treatment is necessary or granules may not be found in the amoebocytes. Both staining reactions show that the granules are neutrophil.

4. Other Methods. The blood was smeared on a slide and fixed with Bouin's fluid, corrosive sublimate, or Flemming's solution and then stained with eosin and Delafield or Heidenhains haematoxylin or picro-indigo-carmine. The blood was collected from the heart, the two kinds of amoebocytes were identified by these methods; but it is very strange that no nucleus could be found in the hyaline amoebocytes. The granules do not stain with eosin, but the cytoplasm stains lightly. I have repeatedly tried to obtain evidence of eosinophilous granules, but without success.

In stained sections the amoebocytes in the organs and tissues are round or oval, with retracted pseudopodia, though very occasionally they are shown expanded. The nucleus is prominent and contains smaller dark bodies (Text-fig. 2 c).

The first description of the granules contained in the amoebocytes of Lamellibranchs is by Lebert and Robin (1846). Subsequently a number of investigators have worked on this subject because their affinity for dyes and their size are important in their classification.

According to Knoll (1893), the granules of amoebocytes of Lamellibranchs vary in the different species with respect to the affinity for dyes; those of Unio, Anodonta, and Solen are acidophil, and Pectunclus is neutrophil. Kollmann (1908) gave a full account of the chromatic leucocyte granules in a number of invertebrates and sought to apply the classification of Ehrlich to the invertebrates. From the results obtained with various coloured substances, he thought that leucocytic granules of the Lamellibranchs are amphophil, with a preference for acid substances. He also divided the properties of the granules of the amoebocytes of Lamellibranchs into two groups according to their habitat; those of fresh-water molluscs are amphophil with a tendency to become stained by acidic dyes, and those of marine molluscs are acidophil. He experimented on the character of the affinity for dyes by making cultures of marine Lamellibranchs in fresh water and vice versa; but he failed to find any change in their affinity for the dyes, and stated, therefore, that these affinities are specific for the different species. If this is true, the granules of the amoebocytes of the oyster which I examined (Ostrea edulis) should be acidophil. However, with several kinds of dyes the granules are not acidophil, they are rather neutrophil like those of Pectunculus (Knoll).

D. Distribution and Origin of the Amoebocytes.

One of the striking features about the alimentary canal of the oyster is the universal presence of large aggregations of amoebocytes around the stomach, intestine, and also other parts. The presence of these amoebocytes is characteristic of most of the Lamallibranchs, and attention has been drawn to their presence by many investigators. Jenkins (1900) observed this to be the case in starved oysters, while Orton (1923) noted their great abundance and widespread distribution in the tissues and particularly round the alimentary canal. He stated that 'It has been noted that this kind of aggregation of blood-cells in a vertebrate would be taken as a sign of inflammation, and that the phenomena in the oyster may have contributed to Pettet's erroneous conclusions about the oyster; but there can be no doubt that in bivalves an aggregation of blood-cells around the gut and especially the stomach is by no means an alarming symptom. The bathing of the gut in the oyster with a fluid containing blood-cells may, therefore, certainly be taken as a normal and necessary condition, and there can be little doubt that one of the functions of the blood-cells is to assist in absorption of food.'

In 1926 Yonge observed in detail the distribution and phagocytic function of the amoebocytes of the oyster, and recognized their great importance in the physiology of digestion in these animals.

Indeed, as shown in a later section, the distribution of the amoebocyte is of the greatest significance in nutrition and excretion in the oyster.

The origin and mode of production of amoebocytes of the oyster are not known; no definite organ is known to produce them. Cuénot (1891, 1914), when discussing conditions in various Lamellibranchs, stated that 'La glande lymphatique est très diffuse; elle est placée à la base de la branchie, mélangée avec les cordons musculaires, le tissu connectif, le nerf branchial, de telle sorte que le sang, en allant respirer, la traverse et entraîne les éléments mûrs qui s'y forment'. Croft (1929) stated that surrounding the whole of the digestive system of Haliotis there is a sheath of connective tissue. This is lymphoid tissue, and contains large cells with blood-cells in all stages of development.

In the oyster, tissue at the base of the gills and the connective tissue surrounding the digestive canal were examined after various methods of fixation and staining, but no evidence could be obtained of any production of amoebocytes under normal conditions.

E. The Functions and Properties of the Granules.

Various hypotheses have been advanced as to the function of the granules of the amoebocytes in invertebrates. They have been considered fat granules, reserve materials, ingested particles, excretory substances, or ferment granules, &c. Cuénot (1891) called the granules of the amoebocytes 'ferment albuminogène ou granules albuminogènes', and he considered that these albuminoid granules were produced by the transformation of the peptone in the digestive tube which poured into the blood after digestion. Kollmann considered them to be actually a reserve albuminoid substance. Canegallo (1923) states that in Anodonta he cannot exclude the idea that some of these granules may be excretory.

The following work was carried out with the view of testing their properties.

(i) Distilled Water.

There is no immediate effect on the granules, they remain intact at the end of thirty minutes.

(ii) Alcohol.

Alcohol (70 per cent., or absolute) does not destroy the granules immediately, but they seem to become gradually coagulated.

(iii) Millon's Reaction.

Lowit (1889) observed the positive reaction of Millon's reagent on the granules of decapod crustaceans, and he concluded that the granules are in the nature of globulins.

No positive results could be obtained with this reagent on the granules of the amoebocytes of the oyster.

(iv) Osmic Acid and Fat Solvent Reagents.

Kollmann studied the chemical nature of the granules of several kinds of amoebocytes in invertebrates and stated that these granules do not react to osmic acid and are also insoluble in ether and other fat solvents. Herdman and Boyce, however, stated that 'With osmic acid a black reaction is occasionally given by the granules'. In my observation, osmic acid (2 per cent.) does not produce any distinct black effect on the granules of the amoebocytes of the oyster, their margins sometimes appear blackish, but never the entire granules. I have repeatedly tried the effects of ether and other fat-solvent reagents on the granules, but they were not dissolved by any of these reagents.

(v) Physiological Diminution of Granules.

Kollmann considered that the granules are reserve material and therefore, when the animal is starved, they diminish gradually as they are utilized by the amoebocytes. He obtained positive results on the amoebocytes of Crustacea (Carcinus).

The number of granules in the amoebocytes of the oyster varies greatly in different cases. Even after ten days' starvation, the granules of the oyster do not decrease to any appreciable extent.

(vi) Sudan III, Scarlet Red.

Canegallo observed fatty globules in the corpuscles of Anodonta by staining with Sudan III; but no such positive results were ever obtained with the amoebocytes of the oyster.

(vii) Dilute Acid and Alkali Solutions.

The actions of dilute hydrochloric acid and acetic acid on the granules are gradual; after a few minutes the granules shrink and afterwards they are destroyed. Dilute sodium hydroxide $\left(\frac{N}{10,000}-\right)$ causes the granules to swell gradually and then to dissolve.

(viii) Bouin's Fluid, Corrosive Sublimate, and Chromic Acid.

Several kinds of fixing fluids for histological processes, such as Bouin's fluid and corrosive sublimate, affect the granules which are gradually destroyed. The cytoplasm which is evenly distributed in the living state becomes a dense network or sponge and sometimes a small number of granules can be found here. If fixation with these fluids is only for a short time, the granules can frequently be identified. Chromic acid (0·5 per cent.) has a moderate effect on the granules which are well fixed by it.

The amoebocyte granules are generally destroyed by these fixing fluids, but such granules as are not destroyed after treatment with them cannot be stained by ordinary dyes such as eosin. This probably explains the absence of granules in the amoebocytes when blood-film preparations are fixed or the fixed sections stained.

(ix) Copper Reaction.

Herdman and Boyce found by histochemical investigation of the green American oyster with 'leucocytosis'[1] that the leucocytes which form the green patch contain a considerable quantity of copper.

Numerous histochemical tests were made with the normal amoebocytes and revealed in some instances faint traces of copper. During the present experiments, a number of oysters suffering from leucocytosis were obtained and then examined for copper. Positive reactions were only obtained with the plasma of the amoebocytes and not with the granules. No copper was ever found in the granules either in healthy amoebocytes or in those suffering from leucocytosis. The occurrence of copper in the plasma of amoebocytes is very interesting and is an important characteristic of the oyster blood.

(x) Temperature.

The effect of temperature on the granules was studied in different degrees of dampness. At about 62° C. the granules gradually coagulate and after a few minutes the cytoplasm which contains the granules assumes the appearance of a dense network.

The preceding results show that the granules of the amoebocytes of the oyster have a comparatively strong resistance to several kinds of reagents. The fat solvents, such as ether, do not dissolve them, and no positive reaction for fat was obtained with Sudan III; but sometimes the margins of the granules become slightly black after treatment with osmic acid. This fact seems to indicate the presence of fat or lipoid substance distributed in this region. The granules have a great affinity for neutral red as already shown. This property is similar to that of the brown cells of the pericardial glands (excretion is discussed later), though the granules of the former are smaller. The granules of the brown cells are, however, not destroyed by various fixations and stain with iron haematoxylin. The properties of these two kinds of granules are thus dissimilar in various respects, and there is no good reason for thinking that the granules represent excrement.

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[1] 'Leucocytosis.' A diseased condition of the oysters in which the heart becomes gorged with whitish green masses of blood-cells was described in some English and American oysters and named leucocytosis by Herdman and Boyce (1899) who could find no other explanation for this than the presence of large amounts of copper in the blood-cells. Subsequently Orton (1923) remarked that the blood-cells of the oysters suffering from the leucocytosis have highly granular and much vacuolated protoplasm, but that they do not appear to be unhealthy.

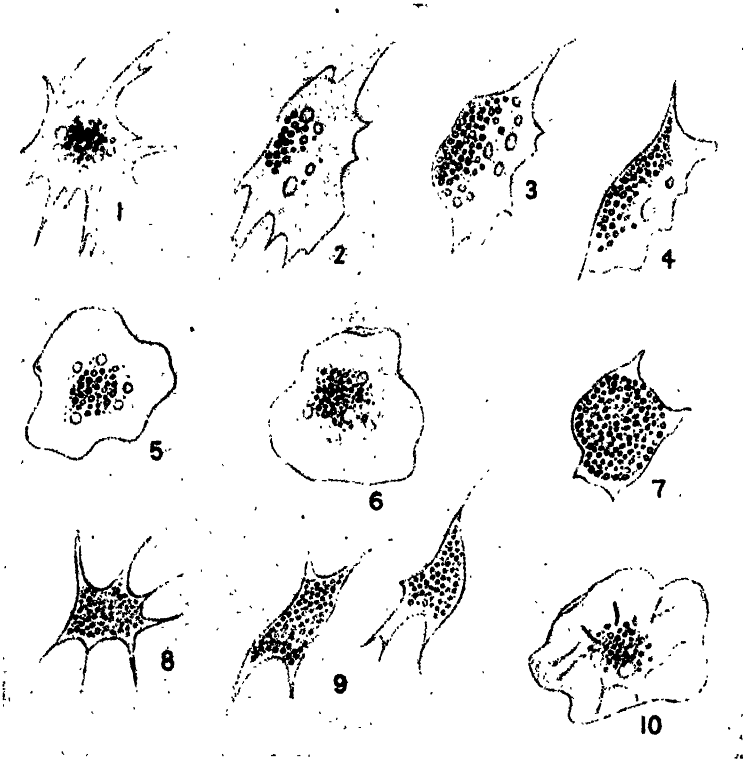
There have been several suggestions as to the coloration of the amoebocytes of the oyster. Ryder (1882) has demonstrated that the green colouring matter of the American oyster is taken up by the amoebocytes and may be phycocyanin. Subsequently Lankester (1886) considered that the green pigments in the gills of the green oyster is concentrated in secretion cells (leucocytes, 1893) and there localized in granules. He studied the solubility of this green pigment, but was unable to dissolve it. MacMunn (1900) thought that the presence of colouring matter in amoebocytes was the result of the decomposition of the ingested chlorophyll which was named by him 'Enterochlorophyll'. Recently Galtsoff (1930) stated that the green pigment of the oyster is readily soluble in methyl alcohol, less so in ethyl alcohol, and insoluble in buthyl or amyl alcohol. It is also insoluble in such fat solvents as chloroform, ether, acetone, or benzene, but soluble in pyridine. He concluded that the green pigment of the body exists in a highly dissociated state.

F. So-called 'Pseudopodia'.

The pseudopodia of the blood-cells of Lamellibranchs have been investigated by many authors, and it is stated that when they come in contact with air or are exposed to changes in the physico-chemical environment they put out pseudopodia. Ryder remarked that the amoebocytes of the oyster put out pseudopodia which may be branched, when at rest. According to Goodrich (1919), the freely projecting pseudopodia of the leucocytes of invertebrates usually described are either figured from optical sections of folded membranes, or due to the changes taking place under abnormal conditions. He studied the leucocytes in many invertebrates, including Ostrea, and concluded that they are provided with more or less extensive membranous processes of cytoplasm, and that in most cases the membranous pseudopodia of the leucocytes are normally expanded in the living animals, but that fine projecting pseudopodia are absent. On the other hand, leucocytes floating in a hanging drop may resemble delicate Heliozoa owing to the appearance of fine radiating processes. This, according to Goodrich, is almost certainly due to the physico-chemical changes taking place in the fluid, and is possibly a sign of approaching death. These fine pseudopodia are probably derived from pre-existing membranes. Recently Fauré-Fremiet (1925, 1928, &c.) and his co-workers studied the amoebocytes of invertebrates in detail by microdissection methods. He called such membranous cytoplasm in the invertebrates 'Pseudopodes Petaloïdes'.

When freshly collected amoebocytes from the oyster are observed, their shape is generally irregular; they congregate by means of the bristle-like pseudopodia (Text-fig. 2 D). Some of them do not congregate, are spherical, and have a distinct ectoplasmic cell membrane. This membrane is very characteristic as recognized by Goodrich; it is a delicate motile membrane which extends around the cell. Careful observations on this motile membrane, and on the so-called 'pseudopodia', reveal the following facts. The bristle-like processes may be derived from this membrane, these processes being the first step in its expansion, or they may also be due to physico-chemical stimulation. Shortly after the amoebocytes have been removed from the animals, the hyaline ectoplasmic membrane begins normally to stretch out in various directions, and the bristle-like pseudopodia become supporting folds in this membrane (Text-fig. 3, 9).

At this stage some pseudopodia-like processes can be observed under a low power; but more careful examination reveals that these 'pseudopodia' are in reality but optical sections of membranous expansions of ectoplasm thinning out peripherally to a very delicate and almost invisible film. Here and there the membranous ectoplasm is strengthened by a rib or folds, which appear as an outstanding process under low power (Text-fig. 3, 10). Sometimes short filamentous pseudopodia are produced accompanied by an expansion of the membranous processes, or the bristle-like pseudopodia gradually expand, being really formed by folding of a single layer of cytoplasm around the cell (Text-fig. 3, 9).



TEXT-FIG. 3.

Various forms which may be assumed by the amoebocytes and their pseudopodia. Drawn from life.

This ectoplasmic membrane can be identified by rapidly fixing the amoebocytes in their expanded condition by means of

Bouin's fluid or corrosive sublimate. It is sometimes very smooth, and sometimes has many folds or ribs, which produce the deceptive appearance of delicate pseudopodia in some fresh material as noted by Goodrich (Text-fig. 3, 10). Generally, the granules are absent in the membranous ectoplasm.

The margin of this membrane is irregular and sometimes shows pseudopodia-like processes which are occasionally branched. This may be related to changes of surface tension. Whenever the membranous cytoplasm comes into contact with a foreign object, as, for instance, a glass slide or a cover-slip, the membranes tend to cling to it and spread over its surface as a thin film (Text-fig. 3, 1 and 2).

The amoebocytes of the oyster are frequently provided with fine filamentous pseudopodia (as figured by Herdman and Boyce). They are probably abnormal, the result of environmental conditions.

In fresh condition the membranous cytoplasm consists of a delicate transparent plasma sheet, and exhibits a plastic rather than viscous flow. Sometimes there are vacuoles, some of which are due to abnormal expansion or may be concerned with intracellular digestion. In fixed stained material it appears reticular or vacuolated. My observations indicate that the delicate membranous sheet is not so expanded in the body as it is outside.

3. PHYSIOLOGY OF THE AMOEBOCYTES.

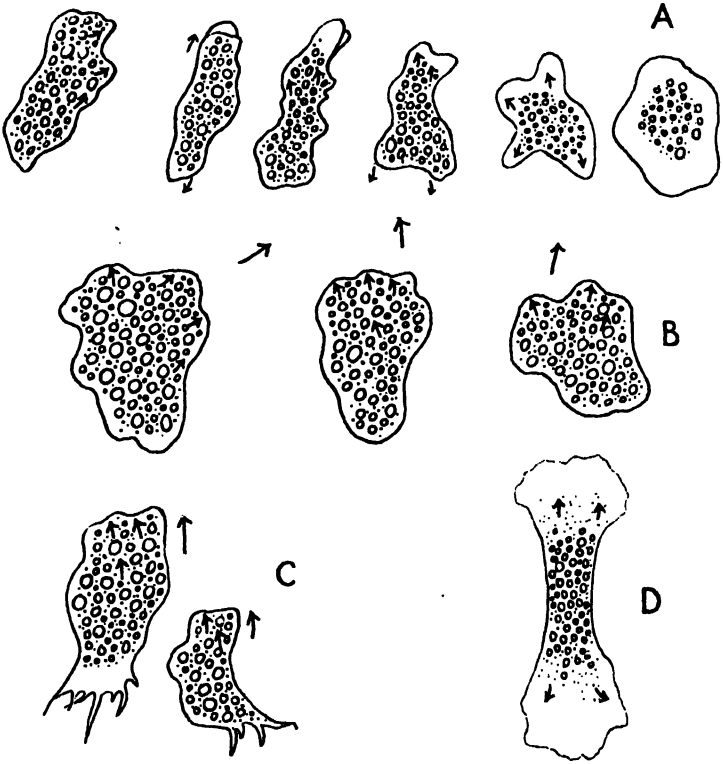
A. Amoeboid Movement.

Many workers have been content to apply the general designation 'amoeboid' to the movements of the blood-cells of invertebrates after they have been taken from the body. Observation shows, however, that the forms of movement manifested by the blood-cells of different invertebrates are not identical. Cattaneo's (1889) observations appear to throw some doubt on the amoeboid characteristics of the ordinary blood-cells of invertebrates. He found that the pseudopodia put out by cells when under observation on a glass slide are not withdrawn. Griesbach (1891) was evidently prepared to admit that there are differences between the movements of molluscan corpuscles and the movements of Amoeba.

The amoeboid movement of the blood-cells of the oyster has been recognized by several authors; Ryder (1882), Lankester (1886), Orton (1923), Yonge (1926), &c. They do not give a detailed description of these movements, but simply refer to them as 'amoeboid' or 'phagocytic'.

The amoeboid movement of the blood-cells of the oyster may be easily seen, although the rate of progression is very slow; unlike those of other invertebrates, such as crustacea, they have a relatively stable constitution. When removed from the body they gradually change in form and, having a relatively high specific gravity, they sink through the plasma or sea-water. The method of movement is variable; the variation may depend upon a number of different factors, such as physico-chemical changes in the environment. The amoeboid movement is due to the so-called 'pseudopodia', which are new processes of clear ectoplasm. Sometimes these are needle-shaped or bristle-like, but more frequently a thin lamella of clear ectoplasm may be seen to flow outwards from all regions along the margin of the amoebocytes. This thin lamella slowly extends when in contact with the surface of the slide. At this stage the cell might almost be likened to a snail emerging from its shell. Loeb (1921) and Tait (1920) consider that this movement is probably due to capillary forces, but it is apparently not entirely so, as the amoeboid behaviour of the cell also seems to have its influence. Now, the direction in which the cell will move depends on the expansion of this external lamella. The part of the external lamella which is in the direction of the forward movement consists of a greater or lesser amount of clear ectoplasm, the granules being usually checked in their forward flow just behind this clear area, although they are carried forward continuously by the endoplasmic stream to the region of the advancing ectoplasm. The main mass of the cell advances to the area previously occupied by the ectoplasm, then a further fringe of ectoplasm extends in the same, or it may be in a new, direction, guiding it still further onwards (Text-fig. 4 A). The glass surface, however, so impedes the movement that active locomotion is difficult.

The expansion of the lamella is not regular. Sometimes two separate lamellae of ectoplasm extend from the opposite poles of the amoebocyte. In this case, each lamella continues to advance, the granular endoplasm becoming extended into a



TEXT-FIG. 4.

Movement of amoebocytes. Drawn from life. A. Progression with so-called pseudopodia. Left to right. B. Progression by flowing of the granules in different directions. C. Amoebocytes showing tail-pieces. D. The expansion of two separate lamellae of ectoplasm from the opposite poles of an amoebocyte.

long leech-like shape as observed by Tait in the blood-cells of the cockroach. The fringe of the ectoplasmic lamella is not similar to the pseudopodia of Amoeba limax. The advancing part spreads outwards like a fan and is probably due to surface tension or thigmotactic action (Text-fig. 4 D).

Pantin (1923) states that in Amoeba limax 'At the hind end was a rugose tail-piece, which with careful observation could be seen to bear a number of fine clear processes, very different from the anterior advancing pseudopodia'. When in motion the hind end of the amoebocytes of the oyster is clear and has a tail-piece less rugose than that of Amoeba. This is probably entirely due to physical differences of retraction of the ectoplasm (Text-fig. 4 C).

This amoeboid movement does not last long. The external lamellae of the cell extend all around the margin of the amoebocytes, but the glass surface so ties them down that free locomotion is impeded. Subsequently the amoebocytes gradually degenerate (Text-fig. 4 A).

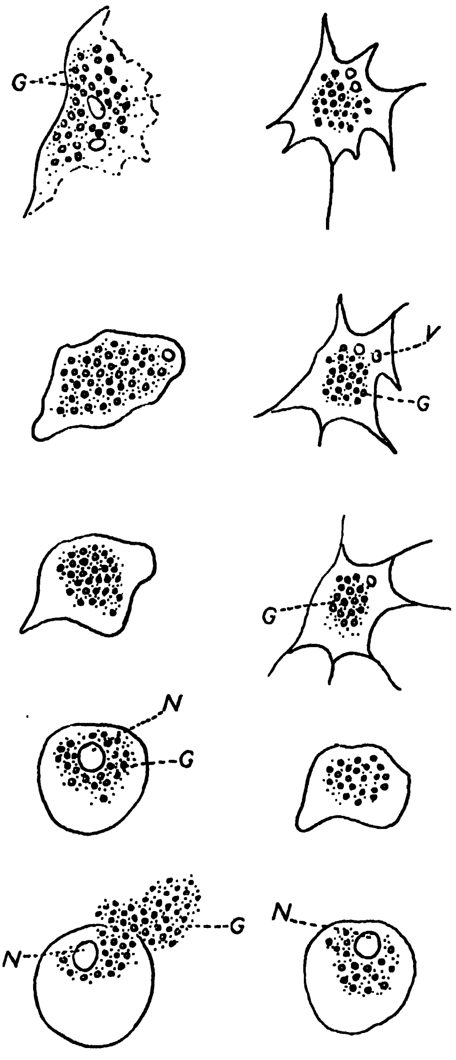
Pantin (1923) considers that the ectoplasm is more acid in the neighbourhood of an active pseudopodium than is the case elsewhere, and concludes that the normal contraction of ectoplasm is due to the production of an acid, leading to an imbibition of water with local swelling. Unfortunately, as previously mentioned, the thin lamella of amoebocytes of the oyster could not be stained with several kinds of intravitam dyes, therefore I could not confirm this.

Sometimes movement appears to consist only of the flowing of the granules in the cell in different directions, and so changing the form of that part of the amoebocyte towards which the granules are flowing. In such cases only a small ectoplasmic lamella is present. I have frequently observed this type of movement in living animals (Text-fig. 4 B).

These two kinds of movement are not distinct from each other. The former type is probably much more affected by environmental conditions than the latter which is probably more normal.

B. Effect of Reagents (Text-fig. 5).

The amoebocytes of the oyster are very sensitive to external conditions. If they are stimulated either mechanically or chemically, the expanded lamellae are retracted and the amoebo-



TEXT-FIG. 5.

Effect of distilled water on amoebocytes. Showing the change of form in two different types. *G.*, granule; *N.*, nucleus; *V.*, vacuole

cytes gradually become spherical. The following experiments were carried out.

(i) Hypotonic Solution; Distilled Water.

The effect of distilled water on the amoebocytes is remarkable; the form gradually changes as shown in Text-fig. 5. They may not immediately undergo cytolysis, but they swell till they are almost spherical. Sometimes the cells, while swollen, undergo a number of violent contractions, ending in cytolysis. The normal process is as follows: the expanded amoebocytes at first become spherical and granules accumulate at the centre of the cell, and then the periphery becomes transparent. At this time the granules show very active Brownian movement, the interior cytoplasm becomes fluid and, when the amoebocytes are becoming spherical, tongue-like processes are produced. This was described by Loeb (1921) as a 'Circus movement' in the amoebocytes of Limulus; it was also observed by Pantin in Amoeba. It is of a purely physical character.

Secondly, the form of the cell becomes much more spherical, and sometimes fine filamentous processes are seen which are the remnants of folds or ribs of the previously existing membrane; they are not processes actually put out from the cell. Thirdly, the cell becomes almost spherical by absorption of water and the granules become distributed eccentrically, the nucleus may be easily identified at this stage, the cell membrane is clearly distinguishable and the cell resembles an ovum with a fertilization membrane. Lastly, in some of them part of the cell breaks down and the granules escape. (The granules are not soluble in distilled water.)

Sea-water diluted with an equal bulk of distilled water produces essentially the same effects, although action is slower.

(ii) Hypertonic Solutions.

In hypertonic solutions water is abstracted from the amoebocytes. Considerable adjustment to the medium is made if the osmotic pressure is changed slowly. In an osmotic pressure twice that of sea-water the amoebocytes gradually change their form, and sometimes large thread-like or filamentous pseudopodia are observed. This is probably due to the physical difference of retraction of the ectoplasmic lamella or possibly to surface tension. The granules move about considerably within the cell which becomes spherical owing to loss of water. Afterwards great shrinkage occurs, though cytolysis does not take place immediately. The cells sometimes resemble the glia cells of the cerebrum because of the thread-like pseudopodia around the margins of the shrunken cell as noted by Loeb.

(iii) Acids, Alkalies, and Fat Solvents.

Under the action of dilute acids, e.g. acetic acid and hydrochloric acid, amoeboid movement is diminished and the nucleus shrinks and becomes more distinct. In certain concentrations a granular precipitate is formed in the cytoplasm around the nucleus. Together with these changes a part of the protoplasm gradually swells out, forming a clear rounded expansion. On the other hand, under the action of alkalies, e.g. by the addition of a very small quantity of caustic soda to the solution containing the amoebocytes, the granules become swollen, and frequently the physical amoeboid movement occurs in the manner demonstrated by Rhumbler and others.

Alcohol and chloroform vapours also arrest amoeboid movement, but it recommences after a time if the action of the reagent is not too prolonged. When gradually warmed the amoebocytes become more and more active up to a certain point (about 35° C.). Above this point they become spherical, and the protoplasm is finally coagulated and killed at about 62° C. At lower temperatures amoeboid movement is also arrested, but although their movement is stopped at about 0° C. they are not killed, and when the temperature is raised movement begins again.

C. Coagulation.

The fact that the blood of Lamellibranchs does not coagulate has long been known. But the amoebocytes usually gather together in irregular clumps and strands. These are the so-called 'plasmodia' described by Geddes (1879), who thought that the cells completely fused and formed a mass comparable to the plasmodia of Myxomycetes. Drew (1910) experimented on the coagulation of the amoebocytes in Cardium blood, and stated that when a wound is made the blood first escapes from it, then the corpuscles begin to agglutinate at the sides of the wound, and connecting strands of protoplasm may be formed between masses of corpuscles and haemorrhage stopped. He also suggested that some change takes place in the corpuscles of the blood which has been withdrawn from the animal, the haemorrhage possibly conferring the power of agglutination on the corpuscles as a result of the liberation of some enzymes from them. He made no attempt, however, to confirm this suggestion. Cuénot (1891), Couvreur (1908), and Camus (1900) commented on the absence of fibrinogen. Nolf (1909) also recognized that there is neither fibrinogen nor thrombine in the blood of certain molluscs.

The amoebocytes of the oyster were allowed to stand after being drawn from the body; the mass of amoebocytes did not become jelly-like by coagulation as in the case of crustacean and vertebrate blood,[1] and no fibrin appears in the blood-plasma which remains fluid on standing in the air and even after being heated. The amoebocytes characteristically become entangled by means of bristle-like psuedopodia or hyaline ectoplasm. When they are accumulated in masses the amoebocytes do not readily fuse together. Indeed those on the edge slowly creep away. The majority of agglutinated amoebocytes do not unite at all, except at the centre where the boundaries of the cells seem gradually to disappear and become more or less structureless, resembling a mass of protoplasm. The amoebocytes which wander away assume various forms; some of them resemble a blood-cell in the act of dividing and become almost separate, until connected by only an extremely fine strand of protoplasm which becomes so fine as to be almost invisible. In certain conditions the amoebocytes will form chains united by long protoplasmic threads. These strands may serve as an entanglement for other corpuscles; they closely resemble strands of fibrin. These structures may act as a plug and so with the contraction of the tissue itself check haemorrhage. The agglutination as described above seems entirely a function of the amoebocytes and not of the blood-plasma. Unfortunately, time has not permitted me to examine the causes of this agglutination.

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[1] Orton (1922) observed that the blood-cells (leucocytes) of the oyster can live for three or four days in sea-water in dishes, and he suggested that it might be possible to cultivate them under appropriate conditions.

D. Phagocytosis.

Phagocytosis[1] by the amoebocytes of Lamellibranchs has attracted the attention of many investigators, and has been experimentally demonstrated by injection or feeding methods (Cuénot, Canegallo, &c.). More detailed experiments were carried out by Yonge (1926) during the course of work on digestion.

The process of phagocytosis has been followed in a variety of ways.

(i) On Glass Slides.

These experiments were carried out with a view to observing the actual process of ingestion (although the environmental conditions were somewhat abnormal). Substances, such as

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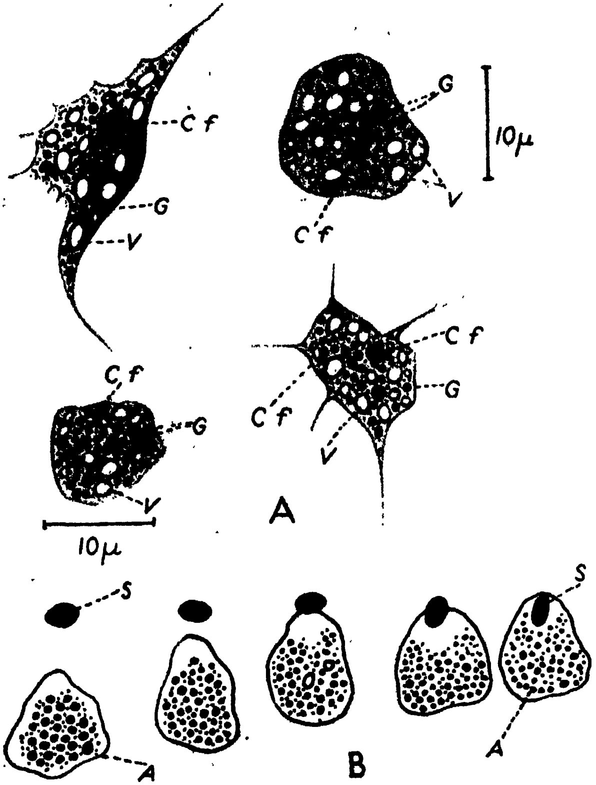
[1] Recently the theory of phagocytosis has received a great deal of attention. Tait (1918) stated that the phagocytic behaviour of the amoebocytes of Ligia may be due to physical causes; accidental contact of particles with the surface of the cell would lead to adhesion. Amoeboid movement is not an essential property of the phagocytic cell. The hyaline thigmocytes of Ligia are not amoeboid, but they engulf particles. Phagocytosis and amoeboid movement are considered different processes by de Haan (1922). The former is 'static' a state of equilibrium produced in the leucocyte under the combined influence of the medium and the object ingested, the leucocyte itself being a non-variable factor; amoeboid movement is a reaction between the leucocyte and the medium, in which the leucocyte exhibits continual variation. Fenn (1920, &c.) made an elaborate investigation of phagocytosis. His fundamental concept was that the taking up of the foreign particles by leucocytes can be explained on purely physical grounds. MacKendrick (1914) considered phagocytosis from a purely mathematical standpoint.

On the other hand, many cases of vertebrate leucocytes taking up foreign organic particles are very probably due to the existence in the blood of an opsonin, a substance which apparently causes the leucocytes to attract particles. Humberger (1919) found blood-serum necessary for the phagocytosis of starch but not of carbon particles. Porges (1909) found that starch is taken up from isotonic solutions without blood-serum as well as with it, but the accelerating effect of blood-serum becomes evident in hypertonic solution. Fenn (1921) stated that there is practically no phagocytosis of carbon particles in the absence of serum or in heated serum. He considered that part of the effect of serum is due to its calcium, since the same amount of Ringers solution also increases the phagocytosis, though to a lesser degree.



TEXT-FIG. 6.

A. Amoebocytes from mantle cavity ingesting carmine fibrin. Drawn from life. *c.f.*, carmine fibrin piece; *G.*, granules of amoebocyte; *V.*, vacuole. B. Amoebocyte attracting a starch grain, and showing the process of ingestion. Drawn from life. *A.*, amoebocyte; *S.*, starch grain.

carmine particles, starch grains, and silver sand, were added to drops of blood on a glass slide, the cover-glass being supported by a small quantity of wax at each corner. After a few minutes in a moist chamber the slides were examined under the microscope. Such an examination is rendered much easier by the fact that the blood as a whole does not coagulate and the amoebocytes move actively in this medium. Examination shows that the amoebocytes certainly ingest particles, although the different particles call forth different reactions. The amoebocytes spread out towards starch grains, to such an extent that they appear to be attracted to them. Sometimes ingestion was assisted by 'pseudopodia'. Ingestion by amoebocytes is a comparatively slow process. The formation of a 'food-cup' or vacuoles preceding ingestion was never seen (Text-fig. 6 B).

Several investigators have observed similar directive movements of the amoebocytes; Drew observed the actual process of phagocytosis of the amoebocytes of Cardium, and stated that the amoebocytes can be seen to send out pseudopodia in the direction of bacteria and engulf them. Agglutinated corpuscles do not appear to possess this power, although motile bacteria in the course of their movements may touch and adhere to them; this is probably a purely passive action on the part of the corpuscles. Commandon (1919) observed that leucocytes of vertebrates are attracted by starch grains. Fenn (1920) found that carbon particles are ingested by leucocytes three or four times more readily than quartz particles of the same size. He also found directive movements of leucocytes towards manganese dioxide particles and stated that the ingestion of such a substance is not due to chance collision. In fact a leucocyte seemed obviously to be attracted, pseudopodia were put out, and one particle ingested.

On the other hand, the amoebocytes are not attracted to silver sand, although after a few minutes this is certainly ingested. This is probably due to chance collision.

When in contact with a glass surface the amoebocytes tend to spread out into a thin film, after which they degenerate. Tait and Ponder (1925) consider that the force which binds the cells to the glass is essentially the same as that which binds the cell to a particle of glass during the preliminary phases of ingestion. Such a force has probably no real connexion with their phagocytic powers as stated by Lison (1929) and Gray (1930).

(ii) In the Test-tube.

Suspensions of amoebocytes and particles of various kinds were kept in a small test-tube for some time, after which a sample was removed and examined on a glass slide. Carmine particles, starch grains, or carmine fibrin pieces are entirely ingested by amoebocytes. In this case the actual process of ingestion by amoebocytes cannot be directly observed. The chance of collision, and so ingestion, in such experiments must be considered in addition to the attraction of particles by amoebocytes.

The results of Yonge's experiments show that amoebocytes digest blood-corpuscles of fish within a comparatively short period after having ingested them. I followed for a long time the process of digestion in the amoebocytes which had ingested carmine fibrin or starch grains, but satisfactory results could not be obtained. The amoebocytes after ingesting particles tend to round up and move away (Text-fig. 6 A).

(iii) Injection.

Several kinds of material, such as carmine particles, Indian ink, starch grains, and olive-oil emulsions, were injected in the body and the oysters then left in an aquarium tank for a given length of time. In this case the amoebocytes become congregated at the place of injection and the particles are ingested. It is probable that the amoebocytes are attracted when foreign substances enter the body in the same way as phagocytes aggregate at foci of infection.

Cuénot observed phagocytosis in the blood-cells of several Lamellibranchs after the injection of Chinese ink. Canegallo (1924) by injecting olive oil stained with Sudan III into Unio, found that this was quickly taken in by the leucocytes. This method admirably demonstrates the ingestion power of the amoebocytes; some of them ingested so many carmine particles that they became red.

(iv) Injection in the Mantle Cavity.

The shells of the oyster, into the mantle cavity of which carmine particles, olive-oil emulsions, &c., were injected, were clamped together to prevent the particles from floating away. After a certain number of hours the amoebocytes were collected from the mantle cavity and examined for signs of phagocytic action. The amoebocytes which wander into this cavity ingested particles, as was frequently observed in natural conditions. Some amoebocytes then passed into the epithelium.

The power of amoebocytes to ingest and digest solid particles is an essential part of the digestion process in the case of Lamellibranchs; ingestion also results in the elimination of waste or foreign particles from the body of the animal. As mentioned in previous experiments, the amoebocytes of the oyster appear to ingest anything that is captured, and there is no evidence of any selection, although ingestion may vary when experiments are made with several kinds of particles. These differences have previously been observed.

Some authors state that when particles are ingested by vertebrate leucocytes they are engulfed in vacuoles from the suspending medium. However, although I have followed the phagocytosis of a great number of particles of different kinds, in all cases they took place by the direct extension of the surface of the amoebocytes (membranous cytoplasm) over the particle. I have never observed ingestion in vacuoles. The membranous cytoplasm of the amoebocytes was in direct contact with the surface of the particle undergoing phagocytosis. Later the ingested particle was entirely enclosed in the endoplasm.

E. Intracellular Digestive Enzymes.

Several investigators have studied the enzyme in molluscan blood. Kobert (1903) found an amylase in the blood of Octopus and a zymase in the blood of Aplysia. Sellier (1906, 1901) recognized a lipase in the blood of Helix, and also in Octopus and Sepia. He also remarked that there is an enzyme which prevents the coagulation of milk. Heymann (1914) found what he called a blood pepsin in the oyster. Recently Sawano (1929) recorded the presence of amylase in the blood of oysters after this had been freed from amoebocytes. None of these workers, however, investigated the digestive power of the phagocytes.

On the other hand, Cuénot (1891) named the granules of amoebocytes of invertebrates 'ferment albuminogène ou granules albuminogènes'. He used the word ferment because it was convenient and on the whole a sufficiently elastic term; he stated that these granules do not form an organized ferment like bacteria or yeast, nor a soluble ferment like a diastase or pepsin; it is only a ferment in a figurative sense. Finally, Yonge (1926) suggested, on the basis of his experiments on the phagocytic action of amoebocytes in the oyster, that 'the phagocytes must also possess powerful digestive enzymes of various kinds'. He identified the lipolytic enzymes in amoebocytes by feeding the oyster with olive-oil emulsions and observing the actual digestion of this and of blood-corpuscles of fish. Graham (1931) recently investigated the digestive enzymes of Ensis, and stated that experiments proved the presence of lipolytic and of amylotic enzymes in the mid-gut and rectum, but they are probably due to the phagocytes which are so numerous in these parts.

The following experiments were undertaken in the hope of determining the nature of the digestive enzymes in the amoebocytes.

The amoebocytes were collected in several ways; when oysters have been heated to about 32° C. in sea-water for some five hours, the amoebocytes may be extruded in numbers on the gill surface or mantle cavity (this phenomenon named 'bleeding' was first described by Orton (1921)). Sometimes another method is employed which was introduced by Yonge (1928) to induce bleeding; the mouths of oysters being plugged by covering the palps with wax held in place with plasticine, this in turn being secured by string tied tightly round the oyster. The oysters were prepared for 'bleeding' by leaving them in the aquarium tanks for one or two weeks, at the end of which period the mantle cavity often contained a great mass of leucocytes. The amoebocytes were collected and preserved in an ice box. After some days the large quantity of amoebocytes accumulated was examined for enzymes. Amoebocytes were also collected directly from the mantle cavity where they are often present in considerable numbers.

In all experiments a water extract of the amoebocytes was used as a source of enzymes; the enzymes were obtained by grinding up the amoebocytes with silver sand, and then extracting with sea-water or distilled water. Throughout these experiments toluol was used as an antiseptic, and rigorous controls consisting of boiled extracts were set up.

Amoebocytes collected in the above-mentioned manner always contain some mucus. According to Gorka (1916) the gill mucus of Anodonta and Unio contains digestive enzymes capable of digesting polysaccharides, glucosides, and fat; and in the mucus of palps he also found a protease. But recently Yonge (1926) tested the mucus of the oyster and was unable to confirm this, although sometimes there was slight enzymatic action, on proteins and fats, which Yonge concluded was probably due to enzymes from the phagocytes.

(i) Action of Extracts of Amoebocytes on Carbohydrates.

The following table gives the results of the principal experi-

TABLE 1. ACTION OF EXTRACT OF AMOEBOCYTES ON CARBOHYDRATES.
Experiment. 2 c.cm. substrate solution + 2 c.cm. extract of amoebocytes.
Control. Ditto boiled.
Temperature. 32° C.

Substrate.	*Time.*	*Result.*	
		Experiment.	*Control.*
1. 1 per cent. starch	2 days	Strong reduction	Very slight reduction.
2. 0·5 per cent. starch	8 hours	Reduction	No reduction.
3. 1 per cent. glycogen	2 days	"	"
4. 0·5 per cent. glycogen	8 hours	"	"
5. 1 per cent. sucrose	2 days	Slight reduction	"
6. " "	20 hours	" "	"
7. 1 per cent. salicine	2 days	Reduction	"
8. " "	20 hours	Slight reduction	"
9. 1 per cent. maltose	2 days	" "	"
10. 1 per cent. lactose	"	" "	"
11. 1 per cent amygdaline	"	Reduction	"
12. " "	20 hours	Slight reduction	"

ments on carbohydrates. Reduction was determined qualitatively by means of Fehling's solution except for the disacharides where Barfoed's solution was used.

Table 1 shows the presence of enzymes which can reduce starch, glycogen, maltose, lactose, and sucrose amongst carbohydrates, and also amygdaline and salicine. Action on starch and glycogen was particularly well-marked. A similar amylotic enzyme has been recognized by several authors in other parts of the body of the oyster such as the crystalline style and digestive diverticula.

It is interesting to note that the glucosides, amygdaline, and salicine are reduced by the extract of amoebocytes, though action is not so strong as on starch and glycogen. It is generally known that enzymes which split these glucosides are widely distributed in the plant kingdom, but do not occur in higher animals. But they have been identified in some invertebrates such as molluscs and crustacea; Yonge found such an enzyme in the digestive diverticula of the oyster. Giaja (1907) demonstrated the presence of one in the hepatopancreas of various decapod crustacea, and Yonge (1924) also found weak enzymes of this type in the hepatopancreas of Nephrops.

Finally, maltose, sucrose, and lactose are slightly reduced by extract of amoebocytes.

(ii) Optimum pH of Sucroclastic Enzymes.

The effect of a change of pH upon the activity of these enzymes is shown in Tables 2 and 3 and Text-fig. 7. The results obtained were uniform, but in all cases the optimum was not so sharply defined as in the case of the enzyme of the style; the efficacy of the enzyme does not decrease so rapidly on either side of the optimum point. As a substrate, a solution of 0·5 per cent. starch or glycogen free from reducing sugar was used. The sugar resulting from the digestion of the substrate was estimated by a modification of the Hagedorn and Jensen method devised by Boyland (1928). This method is satisfactory, as the final titration gives a sharp end-point and so allows accurate results to be obtained. The following mixtures were employed as buffer solutions.

1.	Phthalate—NaOH	pH 4·0	5·6
2.	KH_2PO_4—NaOH	pH 6·0	8·0
3.	H_3BO_3—KCl	pH 8·6	10·0

TABLE 2. THE EFFECT OF pH ON THE ACTION OF THE AMYLASE OF AMOEBOCYTES.

Experiment. 3 c.cm. buffer solution + 3 c.cm. 0·5 per cent. starch solution + 1 c.cm. extract of amoebocytes.
Duration. 30 hours.
Temperature. 28° C.

No.	*Initial* pH.	*Glucose in mg. in* 1 *c.cm.*
1	4·0	0·15
2	4·6	0·25
3	5·0	0·73
4	5·6	0·73
5	6·0	0·73
6	6·4	0·76
7	7·0	0·86
8	7·6	0·71
9	8·0	0·66

TABLE 3. THE EFFECT OF pH ON THE ACTION OF THE GLYCOGENASE OF AMOEBOCYTES.

Experiment. 3 c.cm. buffer solution + 3 c.cm. 0·5 per cent. glycogen solution + 1 c.cm. extract of amoebocytes.
Duration. 30 hours.
Temperature. 28° C.

No.	*Initial* pH.	*Glucose in mg. in* 1 *c.cm.*
1	4	0·18
2	5	0·21
3	6	0·58
4	7	0·60
5	8	0·53
6	9	0·28

The optima of the amylase and glycogenase (which may be the same enzyme) lie at about pH 7·0.

The optimum hydrogen ion concentration for an enzyme reaction often depends upon the type of buffer solution used. According to Michaelis (1912) the optimum reaction for diastase from saliva lies at pH 6·1 to 6·2 in phosphate acetate or sulphate mixtures, but at pH 6·9 in chloride and nitrate mixtures. These differences may be due to the stimulating or inhibiting action of the salts of which the buffer solution is composed.

According to Yonge (1926), starch and glycogen are the only substances acted upon by the enzyme of the crystalline style. There is no action on amygdaline, salicine, maltose, lactose, and sucrose. The optimum pH of the amylase is sharply defined and lies at about 5·9; the efficacy of the enzyme is rapidly reduced, particularly on the acid side. On the other hand, in the enzymes of the digestive diverticula of the oyster he recognized the presence of amylase, glycogenase, maltase, lactase, sucrase, and enzymes which act on amygdaline and salicine. These enzymes are very similar to the enzymes of amoebocytes; but appear to act in a more acid medium. (Optimum pH of the amylase of the digestive diverticula is about pH 5·5.) It appears, therefore, that the amylolytic and glycogenolytic enzymes of the amoebocytes are distinct from those of the crystalline style and digestive diverticula, the optimum pH being about 7·0, and so more on the alkaline side than the other amylases (Text-fig. 7).

(iii) Graham (1931) studied the relation between the optimum pH and the duration of the experiment in the amylolytic enzyme of the crystalline style of Pecten; he stated that 'there is no variations in the pH optimum with variation in the time of the experiment'. Similar experiments were carried out with the amylolytic enzymes from the amoebocytes as shown in Table 4, and similar results were obtained.

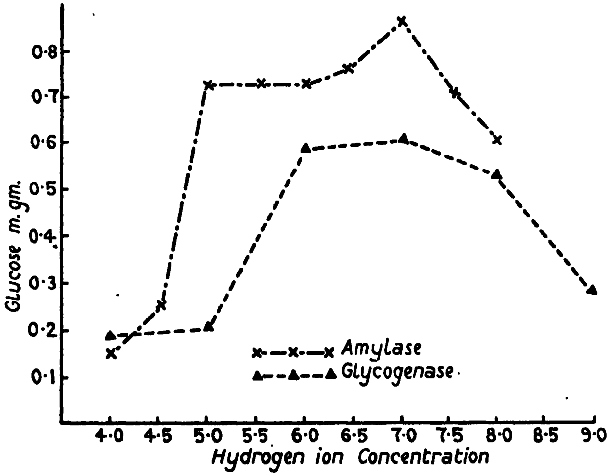
(iv) Lipoclastic Enzymes.

Lipoclastic enzymes of the oyster have been recognized by several investigators; Yonge (1926) found traces in the stomach contents of the oyster, but considered that this originates in the phagocytes, of which many are always present in the stomach. He also recognized the presence of this enzyme in the digestive diverticula of the oyster and stated that 'Since fat is taken in freely by the phagocytes and there digested, very little appearing in the digestive diverticula, there is probably no

TABLE 4.

Experiment. 3 c.cm. buffer solution + 3 c.cm. 0·5 per cent. starch solution + 1 c.cm. extract of amoebocytes.
Temperature. 28° C.

		Glucose in mg. in 1 c.cm.			
No.	*Initial* pH.	*Duration*:	*24 hours.*	*30 hours.*	*54 hours.*
1	4·0	..	0·03	0·15	0·18
2	4·6	..	0·12	0·24	0·89
3	5·0	..	0·25	0·72	2·00
4	5·6	..	0·42	0·73	2·01
5	6·0	..	0·43	0·73	2·20
6	6·4	..	0·44	0·76	2·30
7	7·0	..	0·49	0·86	2·49
8	7·6	..	0·45	0·71	2·02
9	8·0	..	0·43	0·66	1·96



TEXT-FIG. 7.

Based on Tables 3 and 4, the ordinates indicating the glucose in mg. 1 c.cm., the abscissae the pH.

necessity for the presence of a powerful lipase in the digestive diverticula. 'Indeed, the slight lipoclastic action of the extract may be due, in part at any rate, to the phagocytes in the tissue extracted and not to enzymes from the actual absorptive tubules.' He actually demonstrated the presence of a lipoclastic enzyme in the amoebocytes by feeding the oyster with an emulsion of olive oil stained red with Nile blue sulphate.

Several methods were employed for the detection of lipoclastic enzymes. At first an emulsion of olive oil stained with phenol red was used, but as the results were not satisfactory even after three weeks, methyl acetate was used in the later experiments, being finally titrated with sodium hydrate in the presence of phenolphthalein.

The results of typical experiments on the enzymes acting upon fat and ester in the amoebocytes are shown in Table 5.

TABLE 5.

No.	*Duration.*	*Result.*
1. 2 c.cm. extract of amoebocytes; 2 c.cm. olive-oil emulsion	7 days	Slightly yellow.
2. Ditto boiled	,,	Pink.
3. 3 c.cm. extract of amoebocytes; 2 c.cm. olive-oil emulsion	14 days	Slightly yellow.
4. Ditto boiled	,,	Pink.
5. 2 c.cm. extract of amoebocytes; 2 c.cm. 5 per cent. methyl acetate	7 days	Acidity in term of 0·01 N. NaOH 1·7 c.cm.
6. Ditto boiled	,,	0·8 c.cm.
7. 2 c.cm. extract of amoebocytes; 3 c.cm. 5 per cent. methyl acetate	5 days	1·5 c.cm.
8. Ditto boiled	,,	0·5 c.cm.

The results indicate a lipase and an esterase which are probably the same enzyme. This enzyme is not so strong in its action on olive oil. The action of the enzyme is slow and it probably occurs in very small quantities. On the other hand, as mentioned in the feeding experiment, the colour change of the olive-oil emulsion stained with Nile blue sulphate is very distinct.

(v) Proteoclastic Enzymes.

According to Yonge (1926), there are two optima for the proteoclastic enzymes in digestive diverticula; one at a pH of about 3·7 and the other at or above pH 9·0; there is no action at the normal pH of the tissue extract (pH 5·4–6·0), though action takes place at different degrees of alkalinity. He suggested from the above results that one enzyme may come from the digestive diverticula and the other from the phagocytes.

Several experiments were carried out in order to determine the presence of proteoclastic enzymes in the amoebocytes, though on account of the weakness of the enzymes this was not easy.

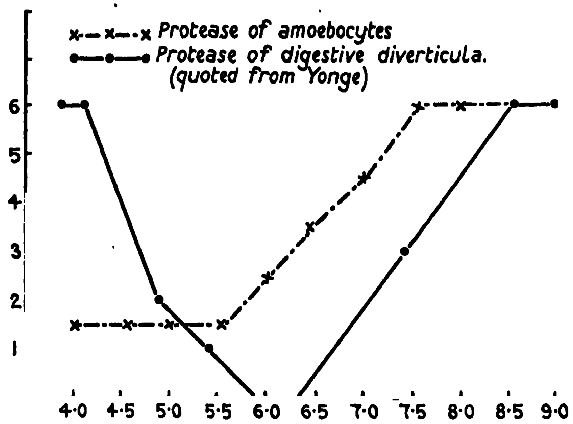
(*a*) Dernby's methods.

The method is briefly as follows: a 10 per cent. solution of gelatine was prepared and a series of experiments performed with extract of amoebocytes at different pH. At stated intervals measured portions (about 5 c.cm.) of the digests were removed. The progress of digestion was determined by placing the digests in an ice box for fifteen minutes, and then observing the degree of liquefaction. Dernby expresses the approximate degree of digestion by means of the following scale of seven numbers. 0 = Completely solid. 1 = Solid, but small pieces may be torn off by strong shaking. 2 = Solid, but the surface moves somewhat when tubes are shaken. 3 = Soft. 4 = Half liquid. 5 = Almost liquid. 6 = Entirely liquid.

TABLE 6. RECORDS THE RESULTS OF A TYPICAL SERIES OF SUCH EXPERIMENTS.

No.	*Initial* pH.	*Control.*	*Degree of Liquefaction at 32° C. for a given Number of Days.*				
			1.	2.	3.	4.	5.
1	4·0	0	1–2	1–2	1–2	1–2	1–2
2	4·6	0	1–2	1–2	1–2	1–2	1–2
3	5·0	0	0–1	0–1	0–1	0–1	1–2
4	5·6	0	0–1	0–1	0–1	0–1	1–2
5	6·0	0	1–2	2–3	2–3	2–3	2–3
6	6·4	0	1–2	3–4	3–4	3–4	3–4
7	7·0	0	2	3–4	3–4	3–4	4–5
8	7·6	0	2	4	4–5	5	6
9	8·0	0	2	5	5–6	6	6
10	9·0	0	5–6	6	6	6	6

It will be observed that digestion occurred only in alkaline media. It is interesting to note that, unlike proteoclastic enzymes of the digestive diverticula, there is only one optimum. This nearly corresponds with one of the optima of the digestive diverticula and so supports the suggestion of Yonge already mentioned (Text-fig. 8).



TEXT-FIG. 8.

Based on Table 6, the ordinates indicating the degree of liquefaction, the abscissae the pH.

(*b*) Digestion of peptone.

In order to determine whether the same condition prevailed, when peptone was used as a substrate an experiment was carried out to determine the production of amino acid at various pH. A 1 per cent. peptone solution was used as a substrate, and for the detection of amino acid Sörensens formaldehyde titration method was employed. The results are shown in Table 7.

As it will be seen in Text-fig. 9 essentially similar results to those already recorded were obtained by this method (Text-fig. 8).

(*c*) Digestion of casein.

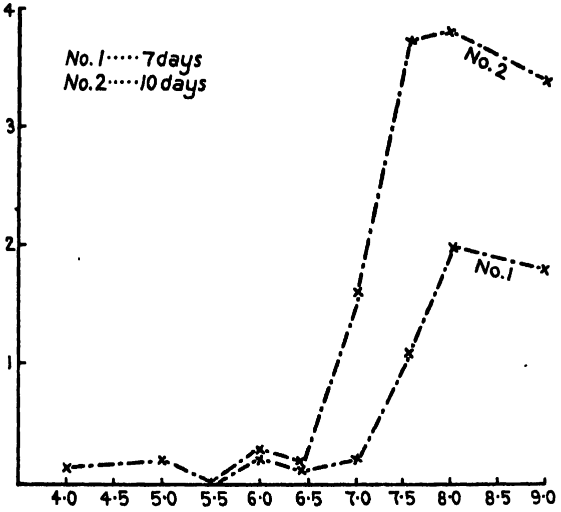
As shown in Table 8, the action of the enzymes on casein is comparatively slight, the pH optimum being obtained on the alkaline side.

TABLE 7.

Experiment. 1 c.cm. extract of amoebocytes + 3 c.cm. peptone (1 per cent.) + 3 c.cm. buffer solution.

Control. Ditto boiled. Temperature. 32° C.

		c.cm. of $\frac{N}{100}$ NaOH *titration:*					
		After 7 *days.*			*After* 10 *days.*		
No. of tubes.	*Initial* pH.	*Cont.*	*Exp.*	*Diff.*	*Cont.*	*Exp.*	*Diff.*
1	4·0	5·4	5·4	0	7·0	7·1	0·1
2	5·0	4·5	4·5	0	5·8	6·0	0·2
3	5·6	3·9	3·9	0	5·2	5·2	0
4	6·0	5·2	5·4	0·2	7·2	7·5	0·3
5	6·4	5·2	5·3	0·1	7·0	7·1	0·1
6	7·0	4·8	5·0	0·2	4·5	6·1	1·6
7	7·6	3·9	5·0	1·1	2·0	5·7	3·7
8	8·0	2·7	4·7	2·0	1·9	5·7	3·8
9	9·0	3·2	5·0	1·8	2·0	5·4	3·4



TEXT-FIG. 9.

Based on Table 7, the ordinates indicating the c.cm. of $\frac{N}{100}$ NaOH, the abscissae the pH.

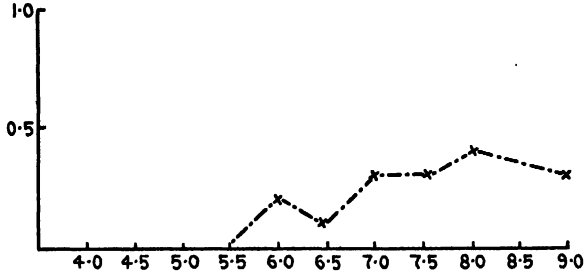
F. Presence of Oxidases.

Jatzenko (1928) observed the reduction of indigo carmine by

TABLE 8.

Experiment. 1 c.cm. extract of amoebocytes + 3 c.cm. buffer solution + 3 c.cm. casein (1 per cent.).
Control. Ditto boiled.

		c.cm. of $\frac{N}{100}$ NaOH: *after 7 days at 32° C.*		
No. of Tube.	*Initial* pH.	*Cont.*	*Exp.*	*Diff.*
1	4·0	7·8	7·8	0
2	5·0	4·6	4·6	0
3	5·6	1·4	1·4	0
4	6·0	3·5	3·7	0·2
5	6·4	5·9	6·0	0·1
6	7·0	4·7	5·0	0·3
7	7·6	3·0	3·3	0·3
8	8·0	2·6	3·0	0·4
9	9·0	2·2	2·5	0·3



TEXT-FIG. 10.

Based on Table 8, the ordinates indicating the c.cm. of $\frac{N}{100}$ NaOH, the abscissae the pH.

the amoebocytes of Anodonta, and concluded that they have the power of taking up oxygen. According to Koch (1917), the blood of Anodonta contains haemocyanin which Jatzenko concluded from his own experiments must be contained in the amoebocytes and not in the plasma.

(i) Indigo Carmine Reduction.

The reduction of indigo carmine by the amoebocytes of the oyster was not rapid and varied considerably.

Experimental solutions, made with 1 c.cm. of pure indigo carmine (saturated) mixed with 10 c.cm. of clean filtered sea-water. Oxygen was first removed as much as possible from these solutions by a vacuum pump, and then an extract of amoebocytes or minced living amoebocytes was added. Then a thick layer of liquid paraffin was added.

The blue colour of indigo carmine gradually became green and then faintly yellow, finally becoming colourless. These reactions took place very gradually, the process being complete in about twenty-four hours at ordinary room temperature (20° C.). Controls were run either without amoebocytes or with boiled amoebocytes. These solutions did not reduce the indigo carmine within twenty-four hours. (The boiled amoebocyte extracts showed a slight reduction.) The minced tissues of the mantle showed no distinguishable reduction.

This reduced indigo carmine did not become oxidized when air was admitted to this solution. The reduction was therefore irreversible. On the other hand, a similar experiment using haemocyanin from crabs was readily reversible, oxidation occurring when air was admitted. The reduction of indigo carmine by haemocyanin took place even after the blood containing it had been coagulated by heat.

(ii) Indo-phenol Reaction.

The indophenol reaction was employed to test for oxidases in the amoebocytes, and positive results in the absence of H_2O_2 were obtained. In a few minutes the solutions containing the extract of amoebocytes become deep purple, other tissues such as minced mantle or labial palps become lighter purple in about twenty minutes. Minced gills gave as rapid reaction as did the extract of amoebocytes. This is probably due to the great number of amoebocytes in the gills. The reaction did not seem to be affected by high temperatures, for activity did not cease after the extract was boiled.

(iii) Guaiacum Reaction.

The reaction of the amoebocytes on guaiacum was very slow and after a day very slight traces of activity were found in the absence of H_2O_2. In the presence of H_2O_2 the reaction was more vigorous, but not so well marked as with the indophenol reaction.

There has been much discussion on the existence of oxidase or peroxidase in the blood of molluscs. Portier and Pieri (1897) found that the reaction of guaiacum on the blood of certain Lamellibranchs produces a blue colour. They also found that an extract of the gills of the oyster was not acted upon by guaiacum with absence of H_2O_2. Subsequently Alsberg (1908) obtained the same results, with the body fluid of the oyster; the reaction was slight, and the results varied in different individuals. He considered that this variation was influenced by the copper content in different individuals. Yonge (1926) also demonstrated that the guaiacum reaction took place very slowly in certain tissues of the oyster.

The results of these experiments on the oyster do not confirm the conclusions of Jatzenko. There is no evidence that haemocyanin is present in the amoebocytes, for the reduction of indigo carmine is apparently due to an oxidase and not to a blood pigment.

G. Absorption.

Absorption in the alimentary canal of the oyster has been followed by Vonk (1924) and Yonge (1926) in detail. Soluble matter such as iron saccharate is absorbed exclusively in the cells of the digestive diverticula (Yonge). Fine particles such as carmine or Indian ink are also taken in phagocytically by the cells of the digestive diverticula (Vonk and Yonge in Ostrea, List in Mytilus), but all larger particles such as droplets of oil, blood-corpuscles, or even such small diatoms as Nitzschia, according to Yonge, are ingested by phagocytes. These are everywhere abundant in the mantle cavity and the gut, but particularly in the stomach, ducts of the digestive diverticula, and in the mid-gut. Particles of any size very rarely enter the tubules of the digestive diverticula, those that enter the ducts being there seized by phagocytes, part of the products of digestion being passed into the cells of the epithelium, and the remainder carried to the vesicular connective tissue. Yonge states emphatically from his experimental results that 'No evidence of any absorption in the epithelium of the gut or of any free surface in the mantle cavity, other than by the agency of phagocytes, was found'.

1. Absorption in Alimentary Canal.

The actual process of absorption in the alimentary canal (including the digestive diverticula) was observed after animals had been fed with various substances such as Indian ink, carmine particles, and olive-oil emulsions, the animals having been previously starved. Various regions of the body were later fixed with Bouin's fluid or 5 per cent. formalin, paraffin or frozen sections being later prepared.

(i) Stomach and Mid-gut.

In oysters opened within one day of feeding, the stomach and mid-gut were full of these substances. In the lumen of the stomach there were a great number of amoebocytes, most of them with ingested particles. Portions of the epithelium of the stomach cleared in glycerine, or after being fixed and sectioned, showed that amoebocytes laden with particles were passing through it. Sometimes a small number of particles not contained in amoebocytes were found in this region. These particles are probably carried in by amoebocytes and not directly absorbed by the epithelium of the stomach itself.

It is impossible, as Yonge has noted, for carmine or Indian ink to be absorbed by the epithelium of the stomach and mid-gut directly, other than by the agency of amoebocytes.

Olive-oil droplets stained red with Nile blue sulphate and ingested by amoebocytes turned a blue colour, but all oil droplets lying free in the lumen in the stomach and mid-gut retained the original red colour. The former is due to the influence of the lipoclastic enzymes in the amoebocytes. The latter provides evidence of the absence of free lipoclastic enzymes in the lumen of the stomach as noted by Yonge in the oyster.

No actual absorption of olive-oil emulsions was found in the ciliated epithelium of stomach or mid-gut other than by the agency of amoebocytes.

(ii) Digestive Diverticula.

Carmine particles and Indian ink are ingested phagocytically by the cells of digestive diverticula. The structure and function of this organ was investigated by Yonge in detail; in brief, the digestive diverticula consist of a brownish mass of blind tubules which surround the stomach and communicates with it by means of two large ducts. These ducts possess cilia and the lumen is irregular owing to variation in height of the epithelium. When examined in sections, cilia are never seen in the tubules. The outline of the cells is indefinite, and their free surface is irregular. They possess phagocytic powers. This organ has been called 'liver', 'hepatopancreas', or 'digestive gland'; but recently Yonge (1926) showed that it is an organ of assimilation and of intracellular digestion with none of the functions of a true liver or pancreas. He therefore suggested that it would be more suitably termed 'digestive diverticula'.

Carmine particles and Indian ink are taken in as round masses in the cells of the tubules and not in a diffuse condition which, according to Yonge, occurs in animals, such as Arthropods and Annelids, in which digestion is extracellular.

After feeding, a small number of droplets of olive oil were observed in the cells of the digestive diverticula, the colour of the Nile blue sulphate in some cases having turned blue as a result of digestion and of the consequent formation of fatty acids.

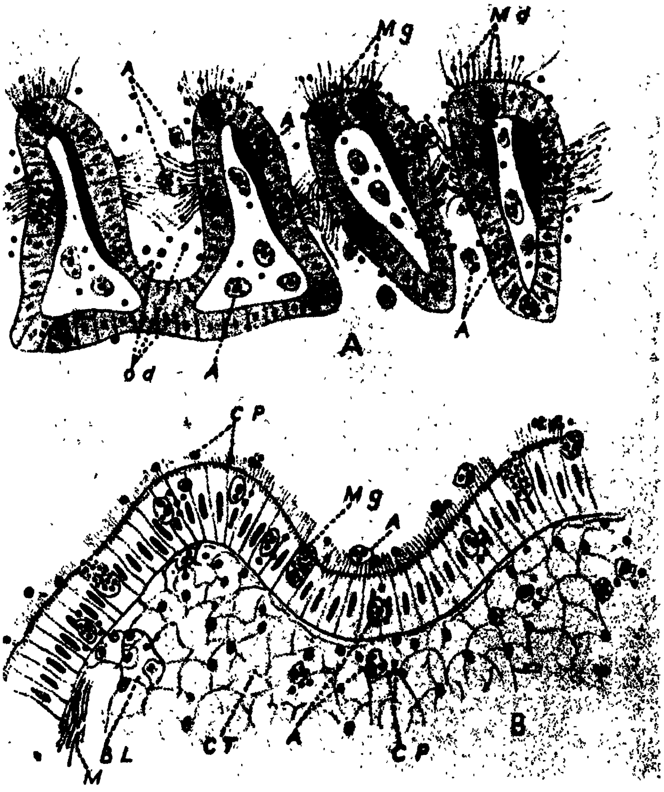
(iii) Rectum.

In the lumen of the rectum there were also many amoebocytes, some of which contained ingested particles, but none of these was seen in the epithelium.

According to my observations, the principal centre of phagocytic activity of the amoebocytes in the gut of the oyster is situated in the stomach and ducts of the digestive diverticula. This conclusion agrees with the finding of Yonge in various Lamellibranchs.

2. Absorption of Substances from the Body Surface (Text-fig. 11 A, B).

The amoebocytes in the mantle cavity ingested particles of carmine or Indian ink, but the degree of ingestion is not great even after long periods (twenty hours to about one month). Those amoebocytes which did ingest particles passed into the tissues. After these had been fixed with Bouin's fluid and then sectioned, wandering amoebocytes with ingested particles were found in various tissues of the mantle cavity. These amoebocytes



Text-fig. 11.

A. Absorption of olive-oil emulsion stained red with Nile blue sulphate by amoebocytes on the surface of the gills. ×450. Freezing method. B. Absorption of carmine particles by amoebocytes on the surface of the labial palps (inner face). ×450. *A.*, amoebocytes; *B.L.*, blood lacunae; *C.T.*, connective tissue; *C.P.*, carmine particle; *M.*, muscle; *M.g.*, mucus gland; *o.d.*, olive-oil droplet.

probably come directly from the mantle cavity where they have ingested the particles. The experiments with olive-oil emulsion stained red with Nile blue sulphate or Sudan III also showed ingestion by amoebocytes which wandered into the tissues. Some parts of the palps and gills were coloured blue as noted already by Yonge.

Sometimes a little carmine, Indian ink, or oil droplets undoubtedly appeared in the epithelium of the gills, palps, &c., after animals had been in a suspension of these particles for several weeks or more. According to Churchill, this may be effected by phagocytic action of the cells. List and Vonk state that the appearance of these particles in the epithelia may be due to the action of leucocytes which had carried them from the digestive diverticula where they are freely absorbed. Hatt (1926), however, states that Indian ink is taken in phagocytically by the epithelium of the gills and palps of Lamellibranchs. This is very questionable; the structure of the epithelial cells of the palps, gills, and mantle provides no evidence of phagocytic behaviour as do the cells of digestive diverticula. The particles appear in these tissues in a diffuse condition, and not accumulated in a round or oval form as seen in the cells of the digestive diverticula. Everything I have observed agrees with the statement of Yonge that in the oyster there is no evidence of direct ingestion of these particles by the body surface and that they are probably deposited there by the phagocytes which either had absorbed them directly from the mantle cavity, or had transported them from the digestive diverticula. An experiment was conducted in which oysters were kept in sea-water containing 0·5 per cent. glucose for five days. The amoebocytes were collected from the mantle cavity and analysed for the presence of sugar by Boyland's method.

Quantity of Sugar in Amoebocytes.

	per cent.	
Experiment . . .	4·4–5·5	3 individuals
Control . . .	2·2–3·5	„

It appears from the above experiments that the amoebocytes readily absorb glucose. This must be greatly facilitated by the very large surface in proportion to the volume.

H. Role of Amoebocytes in Excretion.

The amoebocytes of the oyster take part in excretion as well as in assimilation. The final destination of carmine particles was observed after having been injected into the body and the animal subsequently left in an aquarium tank for a given length of time. The carmine particles were ingested by the amoebocytes and carried to various regions of the body. The final destination of these particles is either in the excretory tubules or in the lumen of the rectum, but the presence of amoebocytes containing ingested carmine was observed in the pericardial epithelium, mantle cavity, and gono-ducts.

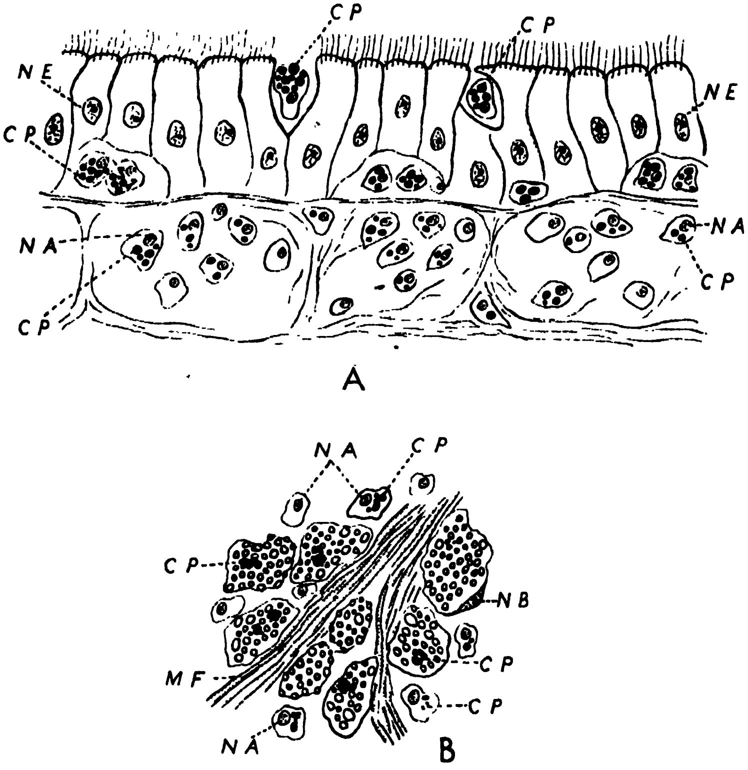
(i) In the Excretory Organ (Text-fig. 12 A).

The amoebocytes containing ingested carmine were observed at several levels in the ciliated epithelium of the excretory tubes about three days after carmine had been injected, but free particles were never observed in the cells of this epithelium. The actual discharge of the carmine particles of the amoebocytes takes place directly, and not by way of the epithelial cells of the excretory organ which never received the particles from the amoebocytes.

(ii) Pericardial Epithelium.

The pericardial epithelium plays an important part in the elimination of waste matter. Many amoebocytes containing carmine particles were found in this region. Some particles are actually taken in by the brownish granular cells. Probably the amoebocytes directly discharge their particles into the pericardial cavity from this region, but some particles are discharged indirectly by way of the granular cells which received the particles from the amoebocytes. The brownish granular cells are amoeboid and behave phagocytically. The same mechanism was observed in the surface of the auricles of the heart (Text-fig. 11 B).

About two weeks after the particles are injected into the body they are found in the pericardial cavity. These particles may, perhaps, be discharged from the pericardial epithelium and auricle surface, either directly from the amoebocytes or, together with other concretions, by way of the brownish granular cells. No amoebocytes were found in this cavity.



TEXT-FIG. 12.

A. Amoebocytes in the epithelium of nephridial tube ingesting carmine particles about three days after these were injected. × 650. *N.A.*, nucleus of amoebocytes; *C.P.*, carmine particles in amoebocytes; *N.E.*, nucleus of epithelial cell. B. Amoebocytes and brown cells in the auricle ingesting carmine particles about three days after these were injected. × 650. *N.A.*, nucleus of amoebocyte; *N.B.*, nucleus of brown cell; *C.P.*, carmine particles; *M.F.*, muscle-fibre.

(iii) In the Gonoducts.

Many amoebocytes which had ingested carmine were found in the gono ducts near the excretory tubules. The reason for the occurrence of the particles in this region is not yet known.

The opening of the excretory organ of the oyster is in the same region as the genital pore, but actually it is situated a little behind this. True communication does not exist between these two openings (Hoek). The particles ingested by amoebocytes found in the gono ducts are directly discharged through these ducts.

(iv) In the Rectum.

About one week after injection free carmine particles were found in the lumen of the rectum. In the epithelium there were many amoebocytes which contained ingested carmine particles, and it is reasonable to assume that the particles in the lumen had been discharged from such amoebocytes.

(v) In the Mantle Cavity and the Blood-vessels.

Amoebocytes with ingested carmine particles were found in the mantle cavity, providing evidence that the ingested particles may be discharged here.

Amoebocytes with ingested carmine are, of course, numerous in the blood-stream before migrating to the regions described above.

4. SUMMARY.

1. There are two kinds of corpuscles in the blood of the oyster; one consists of granular, the other of hyaline amoebocytes.
2. The granular amoebocytes are amoeboid, though the speed of their movement is slow.
3. The granules are yellow or yellowish green in the fresh condition.
4. The granules are neutrophil with a tendency to become stained by the basic dyes intra vitam.
5. Granules can never be distinguished in fixed and stained amoebocytes.
6. The amoebocytes are distributed everywhere throughout

the body, an especially large number being normally present around the gut.

7. The so-called 'pseudopodia' of the amoebocytes are described and their nature discussed.

8. The effects of several kinds of reagents on amoeboid movement and on the amoebocytes are described.

9. The amoebocytes become entangled with one another by bristle-like 'pseudopodia', or by elongated strands of hyaline ectoplasm outside the body. There is no true coagulation of the blood, and no fibrin production.

10. The mechanism of phagocytosis is described and discussed.

11. Sucroclastic, lipoclastic, and proteoclastic enzymes are present in the amoebocytes and enable the amoebocytes to digest intracelluarly.

12. The optimum action of the amylase is about pH 7·0 and of the proteoclastic enzymes about pH 8·0. These optima are not very well marked.

13. A complete oxidase system is present, revealed by the indophenol reaction, by the power of reducing indigo carmine, a process which is irreversible, and by a slight reaction with guaiacum.

14. The amoebocytes can absorb glucose both in the gut and the mantle cavity.

15. There is no evidence of absorption of soluble matter nor of solid substances by the epithelium of the mantle cavity, e.g. gills, labial palps, &c., other than by the agency of amoebocytes.

16. The amoebocytes play a prominent part in excretion. They reject directly foreign or indigestible matter by way of the epithelium of the excretory organ, pericardium, surface of the auricle, rectum, and mantle cavity.

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On Gypsina plana, and on the Systematic Position of the Stromatoporoids.

By

Sydney J. Hickson, F.R.S.

With Plates 26 and 27 and 13 Text-figures.

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INTRODUCTION.

THE study of the collection of large specimens of Gypsina plana (Carter) made by Professor J. Stanley Gardiner in the Indian Ocean, and described by Miss Lindsey in 1913 suggested to me the opinion that there is some relationship between these large Foraminifera and the ancient Stromatoporoids. My recent investigations of specimens collected by Dr. Crossland in Tahiti have so far strengthened and confirmed this opinion that I have no doubt now that the Stromatoporoids were Foraminifera. This conception of the systematic position of the Stromatoporoids is by no means new, as it was held very strongly by W. B. Carpenter and others in the nineteenth century; but as the text-books on Palaeontology still classify these fossils with the Hydrozoa it is necessary to call for a reconsideration of the problem in the light of recent discoveries.

In the course of my investigations Dr. Lang and Dr. Thomas gave me full facilities for the study of the wonderful collection of specimens and slides of Stromatoporoids made by the late Dr. Alleyne Nicholson, now in the British Museum (referred to in the text as B.M.). I have also examined the collection of these fossils in the Sedgwick Museum at Cambridge (S.M.) and in the Manchester Museum (M.M.).

Apart from the question of the bearing of the facts described in this paper on our conception of the systematic position of the Stromatoporoids the study of Gypsina plana has proved to be of considerable interest on account of its great variability—or rather structural adaptation to the variable conditions of life in both shallow and deep sea-waters.

There is no doubt, in my mind, that all the specimens from the Pacific and Indian Oceans, from the Red Sea, and the West Indies belong to the same very variable species, and this may lead to a reconsideration of the validity of the specific distinction of many other forms of sedentary Foraminifera.

I regret that my material was not sufficiently well preserved to give satisfactory results on the nuclei and their mitoses; but Dr. Crossland, to whom I am already greatly indebted for specimens and friendly assistance, has promised to send me, if he can, some specimens from the new Zoological Station at Ghardaqa on the Red Sea, properly preserved for minute histological investigation; and I hope, in a future paper, to be able to describe the nuclear apparatus.

I have had the great advantage of being able to consult Mr. Heron-Allen, F.R.S., and Mr. A. Earland on many occasions. Their wide knowledge of the structure and literature of the Foraminifera, freely placed at my disposal, has proved to be invaluable. I am also indebted to Professor Stanley Gardiner for granting to me facilities for working in the Cambridge laboratory, to Miss Joyce Gardiner for her careful drawings reproduced in Text-figs. 2, 4, and 6, and to Dr. Cowper Reed for assistance with the literature of the Stromatoporoids.

Section 1. STRUCTURE OF THE COENOSTEUM OF GYPSINA

Most of the specimens described in this paper were obtained by Dr. C. C. Crossland in the Society Islands.

Those preserved in spirit are from shallow water on a coral bed off Rahere point in Tahiti, from Rahere reef, and from 15 fathoms at the west end of Papeete harbour.

There are also some pieces of the same Foraminifer attached to the base of a dry coral labelled Acropora pectinata from the reef edge at Tahiti. I have also been able to compare them with specimens from several other localities.

The specimens from Rahere point consist of two dead branches of a Madrepora (Acropora) almost completely covered by the thin white crust of Gypsina. Each branch is about 115 mm. in length by 10–15 mm. in diameter and the crust has an area of 4 or 5 sq. inches on each branch (fig. 1, Pl. 26). The specimen from Rahere reef is roughly cylindrical in form, about 55 mm. in length by 10 mm. in diameter, and the thin white crust completely covers the dead coral support.

As this specimen differs somewhat in character from those from Rahere point it will be referred to as the Rahere reef specimen.

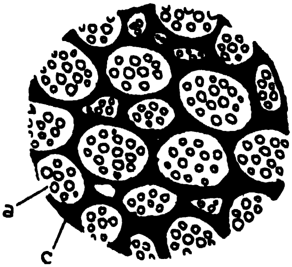
They are all three from shallow water, however, and Dr. Crossland thinks they came from the same locality in which, as a rule, there were strong currents, but that they were sheltered not only by being in the lagoon but also in a more or less closed cranny. As the force of the currents must vary a good deal in such a place it is possible that the specimens from Rahere point were in a more exposed spot than those from Rahere reef, but of this there is no direct evidence.

The specimens from 15 fathoms of water in Papeete harbour consist of several irregular pieces of the Gypsina crust spreading over lumps of rotten coral.

Both the crust and its support are very much more brittle than those of the shallower-water specimens, and there was an accumulation of sandy looking detritus at the bottom of the bottle in which they were packed.

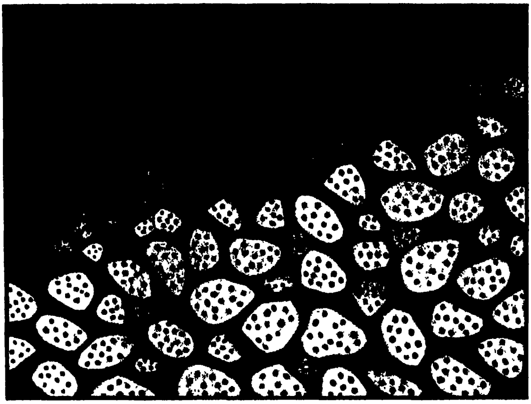
A small sample of this sand was sent to Mr. A. Earland and he sent me a list of over 75 species of Foraminifera that he found in it. I am much indebted to him for this investigation and also for sending me some very beautiful preparations for the microscope of these species.

The Gypsina crust on the coral branch from Rahere point has a smooth surface following the irregularities of the support and has a most striking resemblance to an encrusting calcareous alga. The texture is very hard and compact. According to a note by Crossland it had when alive a 'pink-brown' shade of colour. It was not until I examined a dried fragment of the



TEXT-FIG. 1.

Diagram of a surface view of a specimen from Rahere point, showing *a*, the areolae; *c*, the thick calcareous walls separating them. ×120 diam.



TEXT-FIG. 2.

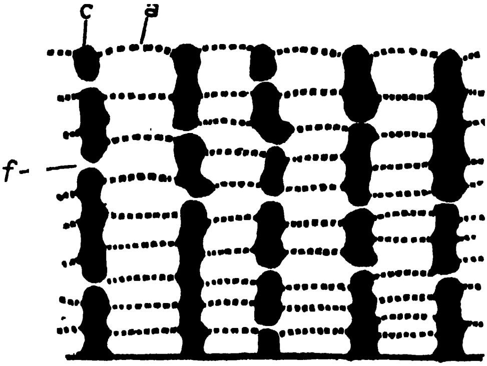
Sketch of a surface view of another specimen from the same locality.

crust with a 1-inch objective that I was able to determine definitely that it is not an alga but a Foraminifer.

The surface under such magnification shows a number of flat perforated areolae bounded by thick solid calcareous walls (fig. 2, Pl. 26; Text-figs. 1 and 2). The areolae vary a good deal in size and shape. Most of them are roughly circular, others pear-shaped, oval, or more irregular. They are from 0·04 to 0·07 mm. in diameter.

The surface appearance resembles very closely that of the Foraminifer Homotrema and my first impression was that it was a white species or variety of that genus.

The structure, as seen in vertical sections or fractures, however, is totally different to that of Homotrema. The crust consists of layer upon layer of chambers arranged in vertical rows with thin flat walls perforated by the pores above and below and thick solid calcareous walls at the sides pierced in places by the foramina which bring the chambers into communication laterally.[1]



TEXT-FIG. 3.

Diagram of a vertical section of a specimen from Rahere point showing *a*, the areolae; *c*, the calcareous walls; *f*, the foramina connecting the chambers laterally.

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[1] In a previous paper (1911) I have referred to the small perforations by which the chambers communicate with the outside world in Polytrema as the 'foramina', a technical error in which I followed several other authors.

In a critical note on the paper of my friend and former pupil, Miss Lindsey, Hofker (1927, p. 9 footnote) has pointed out that strictly speaking the foramen is the larger aperture which connects one chamber with the next in Polythalamous Foraminifera.

There can be no doubt that when D'Orbigny introduced the word 'Foraminifères' he was referring to the apertures between the chambers

As seen in section the chambers are quadrilateral (fig. 3, Pl. 26; Text-fig. 3), the upper and lower sides being perforated and the shorter lateral sides solid.

This structure is similar to that which, by several authors, is considered to be typical for the genus Tinoporus.

The thickness of the crust and the number of chambers in a vertical row vary a great deal. The thickest crust I have measured is 1·15 mm. from the surface to the base of attachment and the number of chambers in a vertical row is 30.

The chambers also vary in size, a rough average is 0·05 mm. in diameter by 0·03 mm. in depth.

The pores of the chambers at the surface of the crust seem to be much larger than they are in many of the perforate Foraminifera. On an areola of 0·07 mm. in diameter there are about 16 pores and these vary from 0·005 to 0·01 mm. in diameter.

A departure from the typical arrangement as just described is seen in some parts of the crust. In places where the surface of the support is depressed to form little cups or crevices and where, therefore, the chambers were more sheltered as they were formed, they are larger, inclined to be oval or spheroidal in form, and have thinner walls.

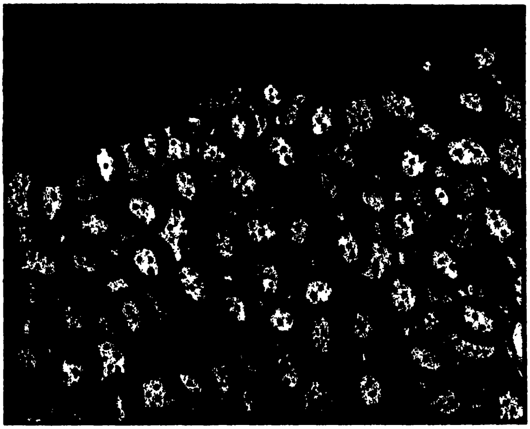
The surface of a fragment of the crust of the specimen from Rahere reef when dried is not so smooth as that of the specimens from Rahere point described above. The areolae are more convex and in some places the connecting tubes (i.e. the foramina) are visible on the surface. They are very variable in size and shape, the largest being about 0·07 mm. in diameter. In a section through the crust of this fragment such a great variety is seen in the size and shape of the chambers that it is difficult to describe it at all adequately. In one place there is an appearance which suggests that there has been a double growth forming two layers separated by narrow spaces. In the lower layer the chambers are in vertical rows. They are about 0·06 mm. in width and only

which have no siphons as they have in his other Order—the 'Cephalopodes Siphonifères'; see Heron-Allen (1917, p. 24).

I have therefore used the word 'pores', as Hofker does, for the small perforations of the shell and 'foramina' for the larger passages between the chambers.

0·015 mm. in depth; in the upper layer the chambers have the same width but are 0·03 mm. in depth. The vertical pillar-like walls of the lower layer are remarkably thick, those of the upper layer are much more slender.

In other parts of this fragment there may be seen thin-walled chambers up to 0·07 mm. in diameter that are oval or spheroidal in shape and not arranged in vertical rows. In fact in such a fragment there may be said to be a condition of the chambers which is intermediate between that of the specimens from Rahere point and of those from deeper water in Papeete harbour.



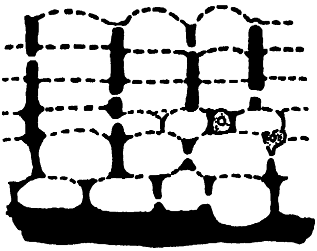
TEXT-FIG. 4.

Sketch of a surface view of a specimen from 15 fathoms in Papeete harbour showing the comparatively thin-walled, dome-shaped areolae projecting from the surface, with, in some cases, the tubular foramina connecting them.

The specimens from deeper water in Papeete harbour have the same general appearance as those described above, but the surface is in most places less smooth and the crust as a whole more brittle.

The areolae stand up from the surface as convex or hemispherical prominences, and short tubes representing the foramina can be seen at the surface bringing the chambers into communication with one another (fig. 4, Pl. 26). In some places the pores of a chamber belonging to the layer immediately below these convex areolae can be seen between them.

In sections or fractures they are sometimes seen to be arranged in vertical rows of three or four chambers above as in the shallow-water forms, resting upon several layers of irregular chambers with thinner walls, which are perforated above and below and are separated from one another by very thin walls perforated by one or more large holes which represent the foramina (Text-fig. 5).



TEXT-FIG. 5.

Diagram of a vertical section of a specimen from Papeete showing irregular spheroidal chambers below and flattened chambers above. The dotted areas indicate the edge of chambers perforated by the foramina.

These thinner chambers are irregularly spheroidal in form and in the mass have a structure very similar to that of a foam.

They are usually larger than the flattened disc-shaped chambers above them; they range from about 0·07 to 0·125 mm. in diameter, with an average of about 0·1 mm., in striking contrast to the chambers of the shallow-water specimen from Rahere point in which the chambers have an average diameter of about 0·055 mm.

In some places these larger chambers extend the whole way from the surface to the base of attachment and there is no arrangement in vertical rows, in others there is an arrangement of chambers in vertical rows above and the larger spheroidal chambers below, the reverse of that seen in the specimen from Rahere reef.

In those parts of the crust in which the spheroidal chambers are at the surface they are seen to be connected by short tubular foramina; but in the lower layers, where the chambers are tightly packed together to form a foam-like structure, the foramina are represented only by small round gaps or holes in the lateral walls (Text-fig. 5).

The foam-like structure of the coenosteum described above, having a resemblance to the arrangement of the chambers in the old genus Acervulina, is usually called 'Acervuline structure'.

It is useful to employ this term to contrast this structure with the Tinoporan structure of the shallow-water specimens.

In this collection of specimens from the Society Islands there is seen a great variety in the structural arrangement of the chambers.

In those from Rahere point the structure is almost typically 'Tinoporan' although some Acervuline chambers are seen in the lowest layer.

In those from Rahere reef there are more Acervuline chambers although the structure is mainly 'Tinoporan'. In the specimens from 15 fathoms in Papeete harbour the chambers are larger and the structure is mainly 'Acervuline' although Tinoporan structure does occur in some places.

It seems probable that these differences in structure are correlated with differences in the environment, the more compact Tinoporan structure being an adaptation to the more exposed positions on the reef, the more fragile and thin-walled Acervuline structure capable of survival in the more placid conditions of deeper water. Corresponding with the change from the Tinoporan type of structure to the Acervuline type there is also an increase in the size of the chambers, the average diameter of the chambers being distinctly greater in the specimens from 15 fathoms than in those from the reef edge.

The view that this difference is really adaptive is confirmed by the structure of specimens from other localities. There are some slides of this species in the Brady collection from Mauritius which were probably collected in shallow water and I have examined other specimens from the Indian Ocean and off Mauritius itself collected by Professor Gardiner at depths of 25, 50, and 100 fathoms.

The specimens in the Brady collection have in great part a typical Tinoporan structure and the chambers are 0·04–0·07 mm.

in diameter. They resemble very closely and in every respect the specimens from the reef edge at Tahiti.

The specimens from 100 fathoms off Mauritius collected by Professor Gardiner are typically Acervuline in structure and have relatively large thin-walled chambers.

The specimens from Amirante (25 fathoms) and Providence (50 fathoms) Islands in the Indian Ocean are intermediate between the two.

The specimen in the Halkyard collection (M.M.) from the West Indies was attached to a shallow-water gasteropod shell and this has the Tinoporan structure with small chambers, and similarly specimens from the Seychelles collected by J. J. Lister with no record of depth have a typically shallow-water type of structure.

Some interesting specimens from the Red Sea were collected and sent to me by Dr. Crossland. They were found at a depth of 3–8 fathoms on a shelf off the edge of the reef near Ghardaqa, growing in the shelter of dead coral but in an exposed place.

When I examined small fragments of these specimens taken from different parts of the very irregular lumps of dead coral which the Gypsina encrusts I found considerable variation in structure. Fragments chipped off prominent knobs were very similar in structure to the shallow-water forms of Tahiti, whereas fragments taken from cavities or holes showed a more brittle structure with a tendency to form Acervuline chambers as in specimens from 15 fathoms off Papeete.

The following table combining the results of Miss Lindsey's (1918) observations and my own is of interest in this connexion.

Diameter of the chambers of Gypsina plana in millimetres.

Rahere point . . .	reef edge	0·04–0·07
Mauritius . . .	shallow water	0·04–0·06
West Indies . . .	shallow water	0·07–0·08
Red Sea . . .	3–8 fathoms	0·04–0·07
Papeete	15 fathoms	0·07–0·127
Amirante . . .	25 fathoms	0·05–0·115
Providence . . .	50–78 fathoms	0·08–0·15
Mauritius . . .	100 fathoms	0·09–0·23

The table shows that in passing from shallow water to deep water the average size of the chambers increases, and this is correlated with a change from a hard compact structure with a smooth surface to a much more brittle consistency with a rougher surface and more exposed chambers in the upper layer.

That it is not entirely a matter of depth is shown by the fact that in several shallow-water specimens from crevices there is an approach to the deep-water characters.

In the depressions on a specimen from the Red Sea, for example, there are clusters of chambers that reach an average size of 0·21 mm. in diameter, and these can be traced into connexion with the chambers of the ridges on a few millimetres away that are only 0·05 in diameter.

The deep-sea specimens, however, are all Acervuline in character.

The deep-sea specimens of Gypsina plana from Mauritius were carefully described by Miss Lindsey (1913). She described and figured the gaps in the walls of the chambers, which correspond with the tubular foramina by which they communicate with one another in shallower-water forms. Unfortunately, owing to the custom of most authors of that time of calling the pores of the perforated plates 'foramina', no clear distinction was made between the larger lateral perforations of the walls, which are the true foramina, and the pores.

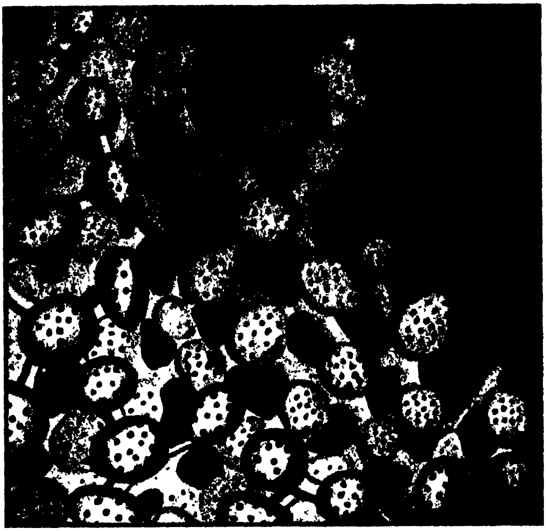
Miss Lindsey did not make a mistake in her description as Hofker (1933, p. 131) asserts, as she called attention to the difference between the larger size of the true foramina by calling them 'gaps in the wall'.

Base of the Crust.

In order to discover, if possible, the embryonic chambers and to determine the question whether the crust arises from one or more fixed embryos, the under-side of several fragments of specimens from different localities was examined.

Hofker (1933, p. 131) states that in a large specimen of this species—the exact size is not mentioned—he found six or seven large embryonic apparatuses of the raspberry type.

After prolonged search I can only say that I have not found a single set of chambers which can with any confidence be called 'embryonic', but on the under-side of the crust there is frequently seen a structural arrangement which is quite distinct from that of the upper layers. This arrangement consists of an open network of chambers connected by tubular foramina and is similar to the open network which is seen at the growing edge of some specimens (see p. 446, and Text-fig. 6). The network consists mainly of moniliform tubes running more or less parallel with one another but frequently branching and sometimes anastomosing. The swollen parts of these tubes are the chambers, the walls of which are perforated with pores corresponding in size and number with those of the chambers of the upper layer.

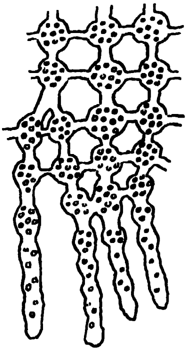


TEXT-FIG. 6.

Drawing of the open network of chambers sometimes seen at the growing edge of a crust. From a Papeete specimen.

In some specimens, particularly in those from the Red Sea, this network is extremely irregular. In some places it extends as a series of plain tubes without any swellings for the chambers, the pores being scattered irregularly along its course; in others the network is very loose, with large gaps, and in one or two regions there may be seen an irregular stellate appearance, the chambers radiating from a single large chamber.

I have found evidence of this network on the under-side of specimens from nearly all the localities including a co-type of the original species described as *Gypsina melobesoides* by Carter (1877) from Mauritius.



TEXT-FIG. 7.

Diagram to show the way in which the network sometimes extends outwards as plain tubes. From a specimen from the Red Sea.

I have not found any reference in the literature to this kind of structure in *Gypsina*, but there is a figure in a paper by Parker and Jones (1865, fig. 2, Pl. xix) of a similar structure at the edge of a small specimen called *Planorbulina retinaculata*. There is no detailed description of this species nor reference to its locality, but judging from the figure only there can be little doubt that it is closely allied to if not identical with *Gypsina plana*.

Growth of the Crust.

The growth of the crust shows some features of special interest. With a low power it seems to advance over the support by sending out thin, irregular, lobate branches which pass round and in some cases encircle various projections, the calices (e.g. of a madrepore) on the support leaving deep bays or sometimes holes in the film. Behind the advancing edges the film thickens, and as it thickens the bases and holes are gradually covered over so that the crust becomes a continuous sheet enveloping the support.

When the edges are examined with a higher power it is seen that the thin film sometimes consists of a number of perforated chambers about 0·06 mm. in diameter at almost equal distances of about 0·03 mm. apart, connected by tubular passages (the foramina) and a thin calcareous membrane joins the chambers and passages together to form a continuous sheet. In some places, however, this thin calcareous membrane is not complete and the growing edge then presents the form of a series of moniliform tubes connected laterally by transverse tubes (fig. 7, Pl. 26; Text-fig. 6). I have not found any evidence that the crust increases in area by fusion at its edges with smaller crusts or embryonic forms.

Behind the thin membranous part of the crust chambers are superposed, at first a little distance apart and joined together by tubular foramina, but later in the thicker part in much greater propinquity and eventually contiguous. It does not seem certain that in the growth of the edge a layer of moniliform tubes, such as has been described above, is always formed because in most of the specimens the edge is relatively thick and composed of typical crowded chambers. Perhaps this form of growth only occurs at times of greater activity.

There seems to be no good reason to deny the possibility that the system of chambers formed from a single embryo may extend over a suitable support almost indefinitely and that specimens of much greater size than any that have yet come to hand will be found.

The resemblance that has been found in some parts of the crust to the structure of Tinoporus and in other parts to the structure of a typical Gypsina might suggest the possibility that it has been formed by a fusion or aggregation of two or more distinct species or even genera.

There is, however, such absolute continuity between the Tinoporus-like parts and the Gypsina-like parts and so many intermediate conditions between them that there can be no doubt that this crust consists of one species only or possibly a group of individuals of the same species in organic continuity.

If there were more than one species there would surely be some break or some indication that the chambers were not organically connected.

Section 2. THE PROTOPLASM.

A general view of the protoplasm may be seen when a part of the crust of a specimen preserved in spirit has been treated with a few drops of strong HCl in about 30 c.c. of 50 per cent. spirit. This is a quick method as it is generally completed in 12 hours.

Far more satisfactory results are obtained if the calcium carbonate is dissolved more slowly in Müller's fluid. This process takes from a month to six weeks.

When the decalcification is too rapid the very thin chitinous lining of the chambers and foramina is broken up and lost. By the slower process a complete but very faint, almost ghost-like, representation in chitin of the calcareous skeleton is left, so that the relation of the hard and soft parts can be easily determined.

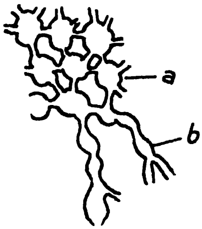
The protoplasm as it is preserved has the character of a moniliform network which may be two or more layers thick. It shows bead-like swellings at the nodes, connected by thin strands with neighbouring nodes and with those of the layers above or below. The nodes evidently correspond with the chambers and the strands with the foramina and pores, but in all the preparations I have examined, from Rahere point, Papeete, and Mauritius, many of the chambers and particularly those of the upper layer are empty.

In a specimen from Rahere point, of the Tinoporan type, many of the chambers of the upper layer are filled with protoplasm (fig. 8, Pl. 26; Text-fig. 8), but there are patches where these chambers are empty and only the chambers of the lower layers are visible.

The network has a different appearance in the lower layers, the nodes being arranged in irregular rows parallel with the surface, connected with one another in each row by long internodes and with only a few connexions with other rows (fig. 9, Pl. 26; Text-fig. 9) and with the other layers. In many places they lose the moniliform character and become plain strands.

In this region, as in the upper layer, many of the skeletal cavities over considerable areas are quite free from protoplasm.

Decalcified specimens from Papeete, 15 fathoms, and Mauritius, 100 fathoms, show a very similar arrangement of the protoplasm, but in the preparations I have made the chambers do not seem to be so well filled as they are in the shallow-water form. But it is in some of these preparations that an irregular star-like appearance of the protoplasm may be seen when viewed from above. From one or sometimes two large nodes at the centre there radiate three or four moniliform or plain strands which after a short course ramify to form an irregular network (fig. 10, Pl. 26; Text-fig. 9).



TEXT-FIG. 8.

Outline sketch of the chambers at the surface (*a*) and their connexion with the more tubular arrangement of the lower layers (*b*); as seen in a decalcified specimen from Rahere point.

This arrangement is of some special interest as it resembles, in a general way, some of the systems of canals which have been described and figured as 'Astrorhizae' in the Stromatoporoids.

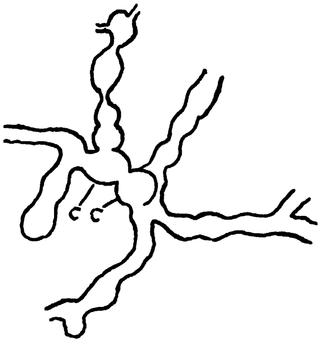
All the cavities are lined internally by a layer of substance, which, without any knowledge of its chemical constitution, may be called chitin. When a specimen is decalcified by the rapid method it disappears but by the more tedious method in Müller's fluid it remains more or less intact.

It is extremely thin in the chambers of the upper and in most of those of the lower layers, but the outlines are clearly retained and the pores of the chamber walls are shown.

The chitin of the peculiar network of tubes and chambers at the base is thicker and traces of it are sometimes seen in preparations made by the more rapid method.

From what has been said above it is evident that the distribution of the protoplasm in these preserved specimens is not what one would expect from a study of the dry skeletal structure.

It may be that during life the protoplasm does not fill the whole set of chambers but flows freely from one region to another in relation to external conditions, or it may be that the arrangement of the protoplasm that is found is partly due to contraction during the time between capture and death in the preservative; but it is very doubtful whether the whole set of chambers is ever completely filled with protoplasm at the same time.



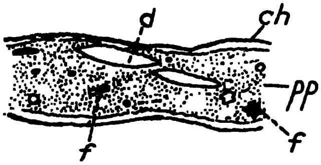
TEXT-FIG. 9.

Outline sketch of an arrangement of the protoplasmic structures at the base with two large chambers in the centre (c) from which four moniliform and tubular processes irregularly radiate; as seen in a specimen from Papeete.

When examined before decalcification the protoplasm is opaque and highly granular, and when dry (fig. 11, Pl. 26) has the appearance of black mud. In decalcified preparations and sections it is more translucent and shows the usual spongy or foamy texture with a high power.

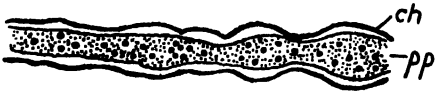
But in many places it is seen to contain granules of sand and other foreign bodies; and among the latter are many frustules of diatoms some of which are 0·035 mm. in length (Text-fig. 10). There can be little doubt that in the process of decalcification many calcareous particles that were in the substance of the protoplasm are dissolved.

The habit of taking into the protoplasm foreign bodies of various kinds is by no means confined to Gypsina among the Foraminifera. In Polytrema and Sporadotrema, for example, it is loaded with fragments of siliceous sponge spicules, grains of sand, diatoms, and other foreign bodies (Hickson, 1911, p. 455).



TEXT-FIG. 10.

A fragment of a tubular structure, showing the chitinous lining (*ch*) of the skeletal structure, the protoplasm (*pp*) and two diatom shells (*d*) and other included foreign bodies (*f*). From a decalcified specimen from Papeete.



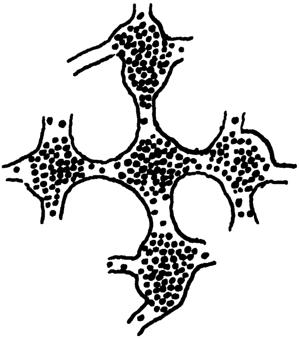
TEXT-FIG. 11.

Another fragment from the same preparation showing a large number of the problematic bodies in the protoplasm. Lettering as in Text-fig. 10.

If a section or fracture of a specimen that has not been preserved in spirit be examined, the protoplasm can be seen to be represented in some of the chambers by a black granular substance (fig. 11, Pl. 26) and this has almost the same pattern as the protoplasm seen when a spirit specimen is decalcified.

A similar pattern of black mud is seen in some beautiful sections of a fossil called Gypsina globulus from Eocene deposits in Hungary, now in the Brady Collection in Cambridge, and I have seen the same thing in sections of Stromatopora hüpschii, Labechia orientalis, and other Stromatoporoids.

In addition to the foreign bodies mentioned later the protoplasm bears numerous small dark granules which, at first, I thought must be nuclei. The chambers of the upper layer containing protoplasm are densely packed with these bodies so that in a whole mount preparation they seem to be filled with nothing but a mass of dark granules. In the strands passing through the foramina they are also numerous but not quite so dense (Text-fig. 12). In the chambers and strands of the lower layers they are not so numerous and in some places widely scattered (Text-figs. 10 and 11). They vary very little in size or shape, being about 3½–4 microns in length by 2 microns in breadth. Experiments with various kinds of staining reagents have failed to provide any evidence of chromatin; in fact, they have a remarkable power of resistance to all the well-known stains. There is no evidence that they divide by direct fission or by mitosis.



TEXT-FIG. 12.

The protoplasm in a group of chambers in the upper layer, showing numerous problematic bodies closely packed together except in the protoplasm of the connecting foramina where they are scattered. From a decalcified preparation of a Rahere point specimen.

Dr. G. Lapage who kindly examined my preparations suggested that they may be reserve food material or possibly excretory matter, comparable with the stercome of other Foraminifera; but there is, in any case, no clear proof that they are nuclei.

Section 3. THE NAME OF THE SPECIES.

There can be no doubt that the specimens belong to the same species as the specimen from 'the shore' at Mauritius recorded but not fully described, by Carter (1877) under the name Gypsina melobesoides. A slide of this species which was probably a fragment of the type was presented by Carter to Brady and is now in the Museum at Cambridge, and I have compared it carefully with the specimens from Tahiti.

But Carter declared that it is identical with a small specimen from South-west Australia which he had previously described as Polytrema plana (1876), and therefore, as Miss Lindsey (1913, p. 50) pointed out, the correct name under the rules of nomenclature is Gypsina plana.

In 1901, Chapman reported the occurrence of a large number of specimens of the same species off the Atoll Funafuti, but unfortunately transferred them back to the genus Polytrema. The two photographs showing the structure, as seen in section, which illustrate Chapman's paper (1901, figs. 6 and 7, Pl. xx), are quite sufficiently clear to prove that his specimens are identical with Carter's specimen from Mauritius.

The reference of the species to the genus Polytrema was unfortunate, and may account for the fact that Miss Lindsey (1913) overlooked Chapman's paper when she wrote her description of the specimens from Mauritius.

The species does not belong to the genus Polytrema. In that genus a constant feature of the structure is the presence of the pillar pores which represent spaces between the chambers opening to the surface by funnel-shaped apertures (0·08 mm. in diameter). Pillar pores do not occur in any specimen of Gypsina plana I have examined, and are not shown in Chapman's figures.

The pores of the chambers are much larger in Gypsina plana than they are in Polytrema. In the former they are simple round holes in a relatively thin shell and vary from 0·005 to 0·02 mm. in diameter; in the latter they are short canals perforating the relatively thick shell, never more than 0·005 mm. at the surface but narrowing to half that diameter where they open into the chambers.

A comparison of the surface of the two forms under a ½-inch objective is, however, far more impressive of the difference than any statement of figures. There are several other points of difference between them, but these characters are quite sufficient to distinguish Gypsina plana from the genus Polytrema.

A more controversial question, perhaps, is whether the species should be retained in the genus Gypsina or given a new generic name.

All the other species of the genus that have been described are very small in size, rarely exceeding 4 mm. in diameter and usually much smaller than that, and they have the appearance of a little heap of chambers more or less round in shape attached to some support; and the surface, showing the convexity of these chambers, is very different in appearance from the smooth areolated surface of the shallow-water forms of Gypsina plana.

If one were to compare a typical Museum specimen of say Gypsina vesicularis with a specimen of Gypsina plana 3 or 4 sq. inches in area it might seem ridiculous to refer them to the same genus.

On the other hand, the surface of the deep-sea forms from Tahiti and from Mauritius agrees in appearance very closely with that of the other species of the genus, and the size and the general arrangement of the pores of the chambers is the same throughout.

Brady (1884, p. 718) stated that in Gypsina 'the chambers have no special stoloniferous orifices and the test exhibits no general aperture'. This character holds good for all the well-preserved specimens of the genus I have examined and constitutes another important distinction between Gypsina and Polytrema.

I have also observed that none of the species of Gypsina possesses the projecting siliceous spicules which form such a conspicuous character of Polytrema, Carpenteria, &c.

The relation of Gypsina to various forms of encrusting Foraminifera which have been referred to the genus Tinoporus is also a matter of controversy.

The type species of this genus is Tinoporus baculatus of de Montfort (1808) and this species, as well as Tinoporus floresianus (see Hofker, 1927, pp. 12, 17) show spines and in some cases projecting columns at the surface. Such spines and projecting columns are not present in any of the species of Gypsina that I have examined. Hofker does not consider that the presence of these spines and projecting columns forms a good generic character because in the microspheric forms they are often not developed at all.

In the definition of the genus Gypsina the same author (p. 8) says that after the formation of the embryonic chambers the later chambers grow without any regularity, but with a tendency to form radiate rows. And in a later passage (p. 9): '"Gypsina" globulus must be named Tinoporus because of its typical arrangement of the chambers in rows. Here lies the—somewhat indefinite—line between the genera Gypsina and Tinoporus.'

On the strength of this definition he refers the specimens attributed to some of the species of Gypsina by previous authors (e.g. Gypsina inhaerens of Parker and Jones, Gypsina globulus of Reuss) to the genus Tinoporus.

In the specimens described in this paper the arrangement of the chambers is so variable that a part of one fragment might, on this character alone, be referred to the genus Tinoporus and another part to Gypsina. A superficial examination of a piece of the shallow-water form from Tahiti would suggest that it is a Tinoporus, but the deep-sea forms from Mauritius would certainly be called Gypsina.

As there is little evidence at present about the characters of the embryonic chambers of Gypsina plana and no means, therefore, of telling the difference between the megalospheric and microspheric forms, the position of the species can only be determined by the characters of the chambers and of the surface taken as a whole. In my opinion, therefore, the species should be retained in Carter's (1877) genus Gypsina.

The characters of the shape, surface, size, and arrangement of the chambers, &c., of Gypsina plana are so variable that the only distinguishing feature of the species is its great size.

The smallest specimen on record is the type specimen described by Carter (1876, p. 211) under the name Polytrema planum which was 6 mm. in diameter. The surface of this specimen, as shown in Carter's figure (fig. 19, Pl. xiii), shows a number of chambers, very irregular in size and shape, and connected by lines (which may be delicate tubes). Judging from this figure, which reminds one of the surface of many parts of the deep-sea forms (fig. 4, Pl. 26; Text-fig. 4), this small specimen differs considerably from other species of Gypsina 2–4 mm. in diameter.

I have not seen any specimen nearly as small as this, although I have searched through the abundant materials in my possession very carefully (see p. 457). We are therefore still in ignorance of the characters of the youngest fixed stages of the species; but it is possible that some of the specimens of Gypsina described under other specific names are only young stages of Gypsina plana. Moreover, there are some specimens in the collections named Gypsina globulus which could not be distinguished from rolled and water-worn fragments of Gypsina plana, and in any case specimens that are not attached to their support and do not show some part of the true surface cannot be identified with any degree of confidence.

The characters which are supposed to distinguish the smaller species of Gypsina from one another do not seem to me at all satisfactory, but we are not in a position at present to assert that they are not good species.

Hofker in his last paper (1933, p. 130) declares that Gypsina plana is identical with Acervulina inhaerens of Schultze (1854) but does not discuss the matter. It is possible that it may be proved in the future that not only Gypsina plana but all the other so-called species of the genus are identical with Schultze's type, and that all of them may have the power, under favourable conditions, of forming encrusting films of relatively great size; but, until that proof is forthcoming, it seems to me very misleading to change the name of a well-known species without good reason.

In 1927 (p. 4) Hofker wrote, 'I believe that the whole genus Gypsina had better be swept away', and, in view of the fact that the character which was supposed to distinguish Tinoporus from Gypsina (i.e. the arrangement of chambers in vertical rows) occurs in the same specimens as the Acervuline arrangement, the species should be given the older generic name

Tinoporus (de Montford, 1808) if a change is to be made at all. But as there is, at present, so much confusion in the systematics of this group of Foraminifera, so many synonyms for every species and probably many redundant and misleading specific names, that a thorough revision, with reference to types, if possible, of all the species is necessary before it can be placed on a sound scientific basis.

Gypsina plana of Carter may be the same species as the very small specimen described and figured by Schultze (1854, p. 68) as the type of Acervulina inhaerens or it may not, but it is certain that the specimens described in this paper are identical with those described as Gypsina melobesoides by Carter because they have been compared with the type or schizotype, and this specific name must, on the grounds of priority, give way to the earlier name Gypsina plana.

Section 4. DISTRIBUTION.

Gypsina plana has a very wide geographical distribution but has hitherto been recorded only from tropical or sub-tropical seas.

Carter's type specimen originally called Polytrema planum came from South-west Australia, and the specimen he called Gypsina melobesoides from the shore at Mauritius.

It is now known for certain to occur in the Society Islands (this paper), at Funafuti in the Pacific Ocean (Chapman), in deep and shallow water off Mauritius, Providence, Amirante, and Salomon and other localities in the Indian Ocean (Gardiner's collection described by Lindsey), in the Gulf of Manaar off the Indian coast (Carter, 1880), in the Malay Archipelago 20–50 fathoms (Hofker, 1933), in the Red Sea (Crossland's collection), off the east coast of Africa in 85 fathoms (Heron-Allen and Earland, 1915), and there is one specimen from the West Indies.

In depth it extends from the reef edge to at least 200 fathoms.

As it is now known to have such a wide distribution it is surprising that there are so few records of its occurrence in the literature on the Foraminifera.

There is no record of it in the Reports on the large collections of Foraminifera made by the 'Challenger', 'Siboga', 'Terra Nova', or 'Discovery' expeditions.

The reason for this may be its large size and the close resemblance that the shallow-water specimens have to calcareous algae.

There are other large Foraminifera such as Carpenteria, Sporadotrema, Orbitolites, Cycloclypeus, &c., but Gypsina plana is the only one that forms thin flat crusts spreading over several square inches of its support.

In Professor Gardiner's collection there are several specimens that are free, rounded or shortly cylindrical in form or more irregular, from 1 to 2 inches in length or diameter. They consist of a core of some coral or other support entirely surrounded by layer upon layer of the Gypsina crusts.

The resemblance of the shallow-water forms to such calcareous algae as Lithophyllum or Lithothamnion is very striking, and the resemblance must be emphasized if other specimens bear the same 'pink-brown' shade of colour when alive that Dr. Crossland noted in his specimens from Rahere point. Other authors have called attention to this superficial resemblance and Carter (1877) gave it the name Gypsina melobesoides for that reason.

Chapman (1901, p. 201) said it is difficult to distinguish from Lithophyllum onkodes.

In my own experience it is often quite impossible to determine whether a given specimen is a Gypsina or an Alga with a lower magnifying power than a 1-inch objective.

There can be little doubt that many specimens have been mistaken for common calcareous algae and cast aside.

Information concerning its distribution in time is lacking, but several species attributed to the genus Gypsina and the closely related Miogypsina are abundant in some of the Tertiary deposits (vide, Vaughan (1924), Thalmann (1932), Caudri (1932), and earlier references).

Section 5. On two small Gypsinas and the Question of the Individuality of the large ones.

It would be important to determine if possible whether these large specimens from 25 mm. in diameter to a size of several square centimetres are single individuals or have been formed by the fusion of several. If each crust is a single individual it should possess one set of embryonic chambers, just as a specimen of Orbitolites, for example, or a large Nummulite has only one set of embryonic chambers. If there are several sets in one crust, that specimen represents as many individuals in organic continuity.

There are many practical difficulties in the search for the embryonic chambers in these specimens. They must be situated on the under-side of the crust and in contact with the support, as in other sedentary Foraminifera, and it is not possible to separate the entire crust from the support without injury to the former.

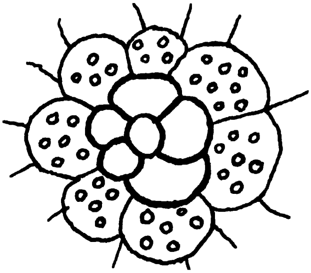
Decalcified preparations do not help in the search, except in the sense of giving negative results, because the distinguishing characters of the chamber walls are destroyed and large areas particularly in the lower layers are found to be free from protoplasm.

At present, notwithstanding a prolonged research, I have been unable to find anything which could be confidently proved to be a set of embryonic chambers in any of the specimens described in former sections of this paper, and I can only assert that there are considerable areas in which there are none.

Hofker (1933, p. 131, fig. 10, Pl. v) states that in a 'large' specimen he found six or seven very large sets of embryonic chambers.

Notwithstanding this discovery of Hofker's I am still of opinion that the large specimens of Gypsina that I have investigated may be single individuals and that there is only one set of embryonic chambers in each of them. If there is more than one individual one would expect to find some indication at their edges of the fusion of two or more independent units, and that I have not found in any specimen from the Society Islands, the Indian Ocean, or the Red Sea. There seems to be no reason why a single individual of this species should not grow to a great size.

In my search for small specimens for the study of the embryonic chambers I found two very small specimens of a Gypsina fixed in the crevices of a very rotten piece of coral from the Red Sea (Text-fig. 13). They are situated on the under-side of a lump which bears on its upper side a large and typical crust of Gypsina plana. They are thin flat discs 0·6 and 0·9 mm. respectively in diameter. The system of embryonic chambers is 0·1 mm. in diameter in each case. They consist of a central chamber or proloculus approximately 0·035 mm. in diameter surrounded by a spiral of five or six chambers gradually increasing in size from 0·035 mm. to a maximum of about 0·05 mm. Outside the set of embryonic chambers, a thin crust of typical thin-walled Acervuline chambers, very variable in size but with an average diameter of 0·08 mm., spreads over the support.



TEXT-FIG. 13.

Sketch of the central part of a very small Gypsina from the Red Sea showing the thick-walled embryonic chambers surrounded by thin-walled Acervuline secondary chambers. × 200 diam.

There can be no doubt that these very small specimens do belong to the genus Gypsina, but there is no clear proof that they are identical with the specimens described in this paper as Gypsina plana.

In other genera of Foraminifera it has been proved that the species have two generations, the megalospheric with a large proloculus and the microspheric with a small one. This may also be the case with Gypsina.

According to Hofker (1927, p. 10, fig. 4, Pl. iii) the proloculus of a small specimen he called Gypsina vesicularis is 0·1 mm. and the whole embryonic 'apparatus' is about 0·3 mm. in diameter. In his later paper (1933, p. 131) the large specimen he called Gypsina inhaerens (but considered by him to be synonymous with Gypsina vesicularis and Gypsina plana) had an embryonic apparatus of about 0·5 mm. and a proloculus of 0·1 mm. in diameter.

According to the same author (1927, p. 7) the proloculus one of the megalospheric forms of Planorbulina larvata is 0·085 mm. in diameter and of the microspheric form 0·03 mm. in diameter.

Comparing these figures it seems that the proloculus of the two small specimens from the Red Sea is smaller than that of Hofker's megalospheric forms of Gypsina inhaerens and Planorbulina, and approximately equal to that of the microspheric form of Planorbulina.

Consequently there is a possibility that the two small specimens are the microspheric forms and that the large crusts may be the megalospheric forms of the same species.

Section 6. THE SYSTEMATIC POSITION OF THE STROMATOPOROIDEA.

The great controversy that took place fifty years ago on the nature of 'Eozoon' of the Archaean age and on the systematic position of the palaeozoic Stromatoporoids gradually died down and is now almost forgotten. It is generally believed to-day that Eozoon is not a fossil but is of purely mineral origin and that the Stromatoporoids are fossil Hydrozoa. In reopening the question of the systematic position of the Stromatoporoids it is necessary to bear in mind that the Eozoon and Stromatoporoid problems were intimately associated in the discussions that took place.

Dawson (1875) and his followers believed that Eozoon was a Foraminifer and that the Stromatoporoids were its linear descendants in the Geologic series. Carter, Nicholson, and many other men of science (see Appendix, p. 476) believed that neither Eozoon nor the Stromatoporoids were Foraminifera.

In reading again many of the papers dealing with this great and often bitter controversy it seems clear that it was not carried on entirely without prejudice. It was, as it were, a skirmish in the greater battle that was in progress on the theory of Evolution.

That the earliest known fossils should be of a simple protoplasmic structure giving rise to calcareous structures similar to those of modern Foraminifera was regarded as a point in favour of the theory of evolution, and this may have influenced some of those who were opposed to the Darwinian views to lend support to the view that the Stromatoporoids were allied to some higher group of animals.

One of the early papers expressing the view that these fossils were Hydrozoa was written by Carter (1878), and he based his conclusion mainly on a comparison between the skeletal structures of Millepora and of Stromatopora. His description of a fragment of a specimen of the recent Millepora alcicornis was not only incomplete but it contained some serious mistakes. He described chitinous canals more or less covered with calcareous material (p. 203) and stated that, not infrequently, the tabula is surmounted by a kind of style—which is indicated in some species of Stromatopora.

In the course of my studies on the genus Millepora from many different localities I have collected a large number of preparations of both hard and soft parts, and I can find no evidence either of chitinous tubes or of styles on the tabulae in any specimen.

In support of his contention Carter also referred to two fossils which he calls Millepora woodwardii and Millepora globularis from the Lower and Upper Chalk respectively. It seems perfectly certain that neither of these species belongs to the genus Millepora or even is related to it.

Millepora is of quite recent origin. There is no evidence that it occurs in any Tertiary or Pretertiary rocks.

In 1897 Professor Zittel kindly lent me a number of fossil corals belonging to the Museum in Münich from the Chalk and other strata which he said were labelled 'Millepora'. As none of these showed the least resemblance to Millepora I published a short paper in 1898 asking for any evidence of the existence of Millepora in Pretertiary times. To that note there has been no answer.

The Stylasterina, a group of Hydrozoa with several distinct genera, seems also to be mainly recent; but at least one genus, Deontopora, occurs in Tertiary rocks. This fossil shows clearly all the essential characters of the group to which it belongs.

Carter (1877), Nicholson (1885, p. 67), and others compared the coenostea of the Stromatoporoids with the skeletal structures formed by some species of the genus Hydractinia, and in a recent paper Tripp (1929) has given a more detailed description of the structure of the hydrorhiza of that and some other hydroids, and pointed out many striking resemblances between it and the coenostea of certain Stromatoporoids.

Whether we are justified in comparing the horny skeleton of these Hydrozoa with what was probably a calcareous skeleton in the Stromatoporoids may be open to some doubt; but a spongy texture of the kind described gives no proof of the Hydroid affinities of the animal that formed it. There are no zooidal tubes and no other indications in this horny substance of the hydroid polyps that live on its surface.

Before passing on to consider the structure of these fossils in greater detail a brief statement may be made as to their distribution in time.

It was formerly believed that the Stromatoporoids were confined to the Palaeozoic period (Eastman, 1900, p. 111) but more recently several fossils referred to this group have been described from Triassic and Jurassic strata.

According to Dehorne (1918) Stromatopora, Stromatoporella, and Actinostroma extend into the secondary series. Yabe (1903) has described a species of Stromatopora from the Jurassic rocks in Japan, and J. Perrin Smith (1927, p. 135) Heterasteridium conglobatum which 'appears to be intermediate between Palaeozoic Stromatoporoidea and recent Hydrocoralla' from the Upper Triassic series.

The group, however, was evidently far more abundant in the Palaeozoic period and appears to have become extinct in the middle of the Mesozoic period.

A very long period, therefore, elapsed between the extinction of the Stromatoporoids and the appearance of fossils that were undoubtedly Hydrozoa.

The numerous genera of fossils which have been included in the Order Stromatoporoidea present considerable variety of structure. In many of them the preservation is so imperfect or the structure so indefinite that there is no clue to their affinities; but in others there are certain features of construction which agree so closely with the characters of Gypsina plana described in this paper that they suggest very strongly close affinities with the Foraminifera.

In the genus Clathrodictyon belonging to the family Actinostromidae, for example, the coenosteum consists of a large number of laminae resting on a support, in a manner similar to that in which Gypsina plana forms an encrusting covering for the dead coral branches.

As seen in vertical section (fig. 12, Pl. 27) each lamina consists of small close-set chambers which are contiguous with the chambers of the laminae above and below. In a preparation of Clathrodictyon striatellum in the Sedgwick Museum these chambers are fairly uniform in size (ca. 0·2 mm. in diameter), oval in shape, and do not show any definite arrangement in vertical rows. In Clathrodictyon variolae (S.M.), Clathrodictyon cellulosum (Nicholson, 1885 p. 78, text fig. 11, Clathrodictyon regulare (Parks, 1933, fig. 7, Pl. i), and others the chambers are more irregular in size, some larger ones being interpolated among the others.

In some species (e.g. Clathrodictyon variole and Clathrodictyon vesiculosum) the arrangement of the chambers as seen in vertical section has a close resemblance to the Acervuline arrangement seen in the deep-sea forms of Gypsina plana (cf. Parks, 1933, figs. 1 and 2, Pl. ii).

In some of the species of Actinostroma the lateral walls of the chambers are thicker and give rise to 'continuous pillars' binding the laminae together (fig. 13, Pl. 27, and Parks, 1933, p. 4, fig. 1, Pl. i) so that a vertical section has a close resemblance to that of a Tinoporus. A somewhat similar arrangement is seen in Hermatostroma Schlüteri, the chambers being in vertical rows supported by pillars (Nicholson, 1883, fig. 2, Pl. iii).

In some other genera (e.g. Chalazodes granulatum Parks, 1907, no. 5, figs. 1–4, Pl. ii, and Stromatocerium rugosum, no. 7, fig. 1, Pl. xxi) the vertical pillars are thicker and more pronounced and sometimes appear to project slightly above the surface of the coenosteum. The chambers are arranged between them in vertical rows giving an appearance very similar to the Tinoporal arrangement of the shallow-water forms of Gypsina.

Many other illustrations of this point in the structure of the Stromatoporoids will be found in the papers by Yabe and Sugiyama (1930) and other authors.

Taking a general view, it is clear that within the Order Stromatoporoidea we find chambers having variations of arrangement comparable to the Acervuline, the Tinoporal, and many intermediate systems of the Foraminifera.

It is obviously an important point to determine whether these chambers were perforated, so as to allow protoplasmic communication between neighbouring chambers and with the laminae above and below, as they are in Gypsina. It is true that perforations of the walls of the chambers cannot be demonstrated in most of the preparations, but in Clathrodictyon cellulosum (fig. 13, Pl. 27) the walls are clearly perforated by large pores and in Rosenella (Parks, 1908, no. 4, fig. 3, Pl. ii) by more numerous and much finer pores. It is probable that other evidence could be found to support the view that the chambers of these Stromatoporoids were perforated; but it is not surprising, considering the changes that must have taken place since they were first fossilized, that in many cases the pores are obscured.

A system of definite chambers arranged in layers is not seen in many of the species of such genera as Stromatopora and Stromatoporella. The coenosteum seems to be perforated by an intricate network of canals without much variation in their diameter.

It is in these forms that the Caunopora and zooidal tubes which penetrate the coenosteum are frequently found.

The Caunopora tubes, as seen in vertical section (fig. 16, Pl. 27), are lined by a relatively thick wall and are usually curved or bent in their course; the zooidal tubes are smaller in diameter, are not usually lined by a wall, and seem to be in communication with the canal system.

Nicholson[1] considered that the Caunopora tubes may be the product of some symbiotic organism, but that the zooidal tubes represent the 'pores' of hydroid polyps, and it was upon the presence of the latter that he based, to a great extent, his opinion on the hydroid affinities of the Stromatoporoids.

A large section of Stromatopora hüpschii (S.M.) shows both kinds of tubes very well. The Caunopora tubes have a diameter of about 0·3 mm. and the zooidal tubes 0·2 mm.; but on tracing one of the former to the surface it is found to lose its inner lining and then differs only from a zooidal tube in its greater diameter, and the impression is gained that the two kinds of tubes were formed by the same or similar symbionts.

There are many reasons for believing that the zooidal tubes do not represent the tabulate pores of a Hydrozoon comparable with Millepora. If they were the tubes of the Hydroid polyps of the Stromatoporoids one would expect to find them in all specimens and in large sections of the coenosteum of the genera to which they belong.

There are two species, Stromatopora dartiana and Stromatopora aporita, recently described by Parks (1933, pp. 19, 20) which seem to belong to the genus Stromatopora in every respect except in the absence of zooidal tubes. There is a section of a species of Stromatopora (S.M.) 10 mm. in length in which there is no trace of zooidal tubes. Stromatoporella is another genus in which zooidal tubes are usually found but in Stromatoporella distincta (Parks, 1933, p. 26) they are apparently absent. Moreover, when present, they are not evenly distributed as one would expect them to be if they were polyp-tubes, but sometimes in clusters, sometimes irregularly scattered at considerable distances apart and with considerable areas entirely free from them.

In comparing the zooidal tubes of Stromatopora with the 'pores' of Millepora, Nicholson (p. 70) laid considerable stress on the presence of tabulae. These structures, however, have proved to be a very untrustworthy guide to zoological affinities. Tabulae are found in some Zoantharia but not in others, they occur in Heliopora and Tubipora, but in no other Alcyonaria; Millepora has a well-developed system of tabulae but most[1] of the genera of Stylasterina have none. In the Foraminifer Sporadotrema mesentericum there is a tabulate appearance in the chitinous canals which penetrate the outer walls of the chambers (Hickson, 1911, p. 459).

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[1] For a full discussion on the 'Nature of Caunopora' see Nicholson, 1885, p. 110 et f.

But, apart from this consideration, the tabulae of the Stromatoporoids differ from those of Millepora in certain important respects. The tabulae of Millepora are always at the same level and have the appearance of being formed simultaneously at that level, probably at the conclusion of a sexual period. Moreover, they are situated at almost exactly equal distances apart in the straight vertical tubes of the gastropores and dactylopores.

In the zooidal tubes of Stromatopora the tabulae, when present, are not on the same level and are situated at very variable distances apart (fig. 15, Pl. 27).

It is quite impossible to assign to the organisms that made the zooidal tubes any definite position in the animal or vegetable kingdom, but their erratic distribution and their general contour suggest that they are not an essential part of the coenostea in which they are found.

Their irregular distribution, their curved forms, and their lack of any special relation to the structures of their host remind one of the way in which the tubes of the Leucodora worm penetrate the colonies of Heliopora. I have examined a large number of specimens of this coral from various reefs, and in every one of them I have found the Leucodora tubes; but sometimes they are very numerous, sometimes scarce, and not infrequently patches of considerable size are free from them.

The zooidal tubes of the Stromatoporoids were not formed by a Leucodora, but there is a strong probability they were formed by a penetrating symbiotic organism.

A more conclusive argument that these tubes were formed by symbionts is afforded by some specimens of Gypsina plana from Salomon Islands (10–14 fathoms) and Centurion bank (5–12 fathoms) in the Indian Ocean. The surface of the crust in specimens from both these localities is seen to be perforated by a number of small holes about 0·12 mm. in diameter. They are quite irregularly scattered; there are some in groups of four or five, others isolated, and there are areas of considerable size entirely free from them. Such an irregular arrangement is quite characteristic also of the Caunoporal and zooidal tubes of the Stromatoporoids.

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[1] Some examples of tabulae in Stylasterina are given by Hickson and England (1909, p. 348).

When seen in a section those tubes in Gypsina show a more or less sinuous course penetrating the crust, and seem to be connected with similar tubes at the base running horizontally. They are very conspicuous among the clear white chambers as they are lined internally by a dark brown layer of chitin. In some places these chitinous tubes show thin irregular transverse or funnel-shaped plates like the tabulae of the zooidal tubes of Stromatopora. In one specimen from Centurion bank and in another from the Red Sea short tubes project from these holes with a length of 2 or 3 mm. and their texture proves almost conclusively that they are worm tubes.

There seems to be very little support now for the view that the so-called 'Zooidal tubes' of the Stromatoporoids correspond with the pores of Millepora and Stylasterina. Steinmann (1903), Parks (1909), and Heinrich (1916) have already opposed it. But, if it is now abandoned, Nicholson's principal reason for referring the Stromatoporoids to the Class Hydrozoa disappears.

The Astrorhizae. In some of the Stromatoporoids stellate structures are found of very variable form and size called Astrorhizae (fig. 18, Pl. 27); but as with the zooidal tubes they are entirely absent from some genera (e.g. Labechia, Nicholson, p. 55) and very variable in distribution in those in which they usually occur. They are common in Actinostroma but very rare in the allied genus Clathrodictyon.

In their most typical form they consist of a central chamber from which radiate a variable number of canals which break up into numerous ramifications to form a network with the branches of neighbouring astrorhizae. In some cases the central chamber is said to be in communication with a vertical tube which gives rise to astrorhizae in lower layers.

Nicholson (p. 55) admits that there does not seem 'to be any ground for regarding these canals as having served for the lodgment of zooids', but nevertheless he compares the astrorhizae with the 'branching and inosculating coenosarcal canals of Millepora' (p. 53).

In my own wide experience of the anatomy of Millepora I have never found any example in the coenosarc that has the remotest resemblance to the typical astrorhizae of the Stromatoporoids.

It is impossible to determine with any degree of certainty what was the nature of the typical astrorhizae, but some of the structures which have been described as astrorhizae (e.g. in Actinostroma stellulatum (Nicholson, 1883, fig. 3, Pl. xiv) have a general resemblance in their form to an arrangement of the chitinous tubes containing the protoplasm which is sometimes found in the lower layers of Gypsina (Text-fig. 9).

In any case their absence from some genera, their extreme variability in others as well as their form, afford no evidence of the relation of the Stromatoporoids to the Hydrozoa.

Carter (1878a, p. 302) refers to the presence of 'chitinous' tubes in certain forms of Stromatopora, and Kirkpatrick (1912, p. 341) has also found certain 'calcified chitinous' rings in the canals of Stromatopora and Actinostroma. I have not been able to confirm these observations; but, if it can be proved that the cavities are lined with chitin, it would constitute a strong point in favour of the view, which Kirkpatrick supports, that the Stromatoporoids were Foraminifera, because such a chitinous lining is found in all the large Foraminifera and never in Millepora nor in the Stylasterina.

The two kinds of Hydrozoa which form massive calcareous skeletons and might, had they been in existence, have become fossilized and preserved from Palaeozoic times onwards are the Milleporina and Stylasterina, usually, but unwisely, grouped together as the Hydrocorallinae (Hickson and England, 1905, p. 2, and Hickson, 1924, p. 150).

They agree in showing dimorphism of the polyps and also of the tubes—the so-called gasteropores and dactylopores—in which the polyps reside. Between the pores the coenosteum is perforated by a network of canals which in life contain or formerly contained the coenosarcal canals.

In Millepora—the only genus of the Milleporina—the tubular pores are numerous, evenly distributed over the branches or foliate upgrowths, passing vertically to the centre of branches, and parallel with one another. They never branch nor anastomose. The zooidal tubes of the Stromatoporoids are not dimorphic, are very sporadic in distribution, are not parallel with one another, and are sinuous in form. They frequently branch and anastomose. The tabulae of Millepora as indicated above (p. 466) are very different in character from those of the zooidal tubes.

In the Stylasterina the gasteropores and in some genera both gasteropores and dactylopores bear styles, characteristic structures which are preserved in the few Tertiary fossils belonging to this order. The pores as they penetrate the coenosteum are often curved but never sinuous, and very rarely exhibit tabulae. The ampullae which bear the gonads are large cavities in the coenosteum, and form a very conspicuous feature at the surface of nearly every specimen in all the genera. None of the Stromatoporoids shows a group of characters at all comparable with those of the Stylasterina.

There is no Pretertiary fossil that shows any of these characteristic features.

The view that the Stromatoporoids were closely related to if not actually Foraminifera receives support from the character of the inclusions seen in the chambers and canals of many of the species.

It is a character of the protoplasm of Gypsina and some other Foraminifera (see p. 450) that it bears a large number of foreign granules, so that it has sometimes the appearance of a black mud. The endoderm of the polyps and the canals in the calcareous Hydrozoa never contain foreign substances of this kind. Dead and dry corals of this group do not show muddy infiltrations into the cavities. In many of the Stromatoporoids some of the cavities are frequently found to be filled with a coloured granular substance. Nicholson (p. 32), for example, refers to a fine-grained mud filling the cavities in Labechia ohioensis and Labechia canadensis and gives a figure (fig. 1, Pl. iv) of the astrorhizae of Stromatopora dartingtonienses 'filled with a more or less opaque calcareous mud' (p. 202).

In a slide of Stromatopora hüpschii (S.M.) the chambers at the upper edge are filled with a dark orange-coloured substance which forms a pattern almost exactly like that of the protoplasm in a decalcified Gypsina. In three species of the same genus from the Devonian rocks at Teignmouth (M.M.) the granular substance is of a reddish colour, and I have seen the same kind of substance in specimens in the British Museum collection of Clathrodictyon, Stachyodes, Hermatostroma, and Labechia.

This substance is not distributed evenly throughout the coenostea and when confined to the upper layers it is usually in patches, the lower layers of chambers and most of the chambers of the upper layers being empty or filled with calcite crystal.

This distribution in patches is comparable with that of the protoplasm in Gypsina (p. 447, and fig. 11, Pl. 26).

The system of chambers in the composition of the coenosteum of many genera such as Clathrodictyon, Actinostroma, &c., suggests at once some affinity with the Foraminifera. It is quite unlike anything that is found in recent calcareous Hydrozoa or in the horny skeleton of Hydractinia. If the chambers were for the protection of the coenosarcal canals of a Hydrozoan colony, the polyps must have been entirely superficial to them as they are to the skeleton in Hydractinia; but there were no definite apertures or passages by which the canal system could be brought into communication with the polyps.

The chambers were in communication with one another, in some cases, by fairly large pores as they were in Clathrodictyon cellulosum (fig. 14, Pl. 27) or by very numerous fine pores as in Rosenella (see p. 464). Such pores are comparable with the larger and smaller perforations of the chamber walls of Gypsina and Polytrema, but there is nothing comparable with them in the Hydrozoa.

A glance at a number of illustrations of vertical sections of

Stromatoporoids by various authors shows that the chambers are not uniform in size, but exhibit variations in size and shape remarkably similar to those seen in a vertical section of Gypsina plana. Moreover, in different genera we find the Tinoporan, the Acervuline and intermediate arrangements of the chambers as we find them in the Foraminifera.

The Idiostromidae. The fossils which are usually collected together to form the Order Stromatoporoidea show such a wide range of form and structure that doubts have been expressed as to whether it is a monophyletic group.

Heinrich (1916), for example, considered that the family Idiostromidae, which includes the genera Stachyodes, Amphipora, and Idiostroma, is incorrectly referred to the Stromatoporoidea and should constitute a distinct group. It is interesting, therefore, to consider separately the structure of these genera to see if they show any better characters to justify their inclusion in the Hydrozoa than the Actinostromidae.

All three genera have a very different habit to such genera as Actinostroma, being cylindrical and dendritic instead of laminar and encrusting.

In Stachyodes the coenostea are rounded cylindrical stems, occasionally bifurcated or feebly branched distally, rooted basally, and terminating in rounded ends.

Nicholson (p. 71) compares them with the coenostea of the Stylasterine Distichopora although they never show anything corresponding with the dimorphic pores, the styles, or the ampullae of that genus. Moreover, the coenostea of Distichopora and all the other Stylasterina are far more freely branched, and in most of the genera the terminal branches are very delicate. In external features it has a much closer resemblance to the cylindrical feebly branched specimens of Sporadotrema (Hickson, 1911).

In section a branch of Stachyodes (fig. 17, Pl. 27) Nicholson shows an axial tube with irregular and sometimes funnel-shaped tabulae. The 'tube' does not open directly to the exterior at the end of the branch, but, in some cases at least, divides into a number of smaller tubes which open to the exterior. There can be no doubt that this is not a 'zooidal' tube comparable with a gasteropore of Distichopora or any other Stylasterine. From this tube there branch off a number of smaller tubes which Nicholson calls the zooidal tubes and these subdivide to open eventually to the exterior as minute pores.

Amphipora consists of slender cylindrical tubes which sometimes branch dichotomously, and Idiostroma also consists of cylindrical tubes 'which branch and inosculate freely so as to give rise to fasciculate masses of considerable size'. Both of these genera exhibit axial tubes with irregular tabulae (see Nicholson, p. 226, Text-fig. 32, and fig. 1, Pl. ix).

The general external appearance of the masses of branching tubes which specimens of these genera exhibit call to mind the great conglomerates of Ramulina herdmani that are found on the sea bottom off Ceylon and in the Malay archipelago.

Ramulina consists of a number of vesiculate chambers, called by Dakin the 'ampullae', which give rise to tubular projections called the 'pipes'. I have not seen anything in the specimens of Amphipora and Idiostroma I have examined that exactly corresponds with the 'ampullae' of Ramulina, but the 'pipes' have a very close resemblance to the cylindrical tubes of these Stromatoporoids.

The surface of the pipes is, in some places, perforated by pores and in others it is covered by a 'thin imperforate calcareous membrane' as it is in Amphipora (Nicholson, p. 224) in Stachyodes and in Idiostroma. The lumen of the tube of Ramulina frequently shows calcareous and chitinous membranes and fibres which have a general resemblance to the irregular tabulae which are seen in section, of all these genera. The resemblance between some of the branches of Amphipora and the 'pipes' of Ramulina is really very striking.

The axial tubes which form a constant characteristic feature of these Stromatoporoids seem rather more difficult to account for. A clue to their significance, however, may be found in the genus Beatricea. In this genus 'the axial tube has no definite walls, but is formed by the superposition of deeply convex vesicles of large size, the convexities of which are all turned in one direction' (Nicholson, p. 87, fig. 3, Pl. viii).

These vesicles correspond very closely in appearance with the thin-walled Acervuline chambers enclosed within the thick walls of Sporadotrema (Hickson, 1911, fig. 17, Pl. xxxi).

If the axial tubes of the Idiostromidae are homologous with that of Beatricea they may represent rows of large, centrally placed, thin-walled chambers of the Foraminifera. It might have been argued that a row of chambers cannot be homologous with an open axial tube, but we have seen that at the growing edge of Gypsina plana a row of chambers is actually continuous with plain open tubes (p. 445, Text-fig. 7), and there seems to me no reason to doubt that the axial tubes of the Idiostromidae do correspond with the more or less tubular arrangement of the chambers often seen in the basal layer of Gypsina plana.

The 'microscopic tubuli of the skeleton fibre' of Stachyodes have been compared with the network of coenosarcal canals which connect the zooidal tubes in Distichopora and other Stylasterines. I have examined sections and other preparations of Distichopora, Stylaster, and several other genera of Stylasterina in my possession, and compared them with Nicholson's sections of Stachyodes in the British Museum, and have formed the opinion that this is a 'false homology'. The coenosarcal canals of Stylasterina form a plain open network in three dimensions and the principal meshes run more or less parallel with the surface; the canal systems of Stachyodes are branching tubuli passing at right angles from the axial tubes to the surface. They have much more resemblance to the canal system that passes from the central chambers through the thick walls to the surface in Sporadotrema and other Foraminifera (see Hickson, 1911, fig. 17, Pl. xxxi, and Hofker, 1930, fig. 1, Pl. lii) than to any system of canals in the calcareous Hydrozoa.

Taking a general survey of the characters of the Idiostromidae there seems to be no reason to accept Nicholson's view that they were related to the recent Stylasteridae, or that they even represent 'an ancient type of the Stylasteridae'.

If they were calcareous Hydrozoa one would expect to find distinct evidence of zooidal tubes in all or nearly all the specimens. They need not be dimorphic, as 'an ancient type' might have been monomorphic. But the so-called 'zooidal tubes' which occur spasmodically in some of the Idiostromidae are essentially different from the gasteropores and dactylopores of Millepora and the Stylasteridae.

Moreover, in the latter the pores are in definite relation to a network of coenosarcal canals which are free from extraneous inclusions, whereas in the Idiostromidae the canal system seems to have no relation to the zooidal tubes, and is frequently loaded with 'mud'.

There seems in fact to be no definite character by which they show any clear relationship with the Hydrozoa.

There are several other fossils which are still included in the Class Hydrozoa but transferred to the Family Tubulariidae (Eastman, p. 110).

Among these is the genus Parkeria, a very common fossil in the Cambridge Greensand of which I have had the opportunity to examine several specimens and preparations. The genus was well described by Carpenter and Brady (1849) and I can find no good reason to dissent from their view that it was an arenaceous Foraminifer.

Parkeria, as well as Ellipsactinia, Loftusia, and other genera included in this family, are usually globular or walnut-shaped fossils consisting of concentric layers of calcareous laminae growing round a central foreign body.

There is no recent Tubularian which has the least resemblance to this structure; in fact it is difficult to believe that any Hydroid could assume a spherical form liable to be rolled about by the sea currents and continue to survive.

On the other hand there are several specimens of Gypsina plana, some an inch or more in diameter, in Professor Gardiner's collection from deep water in the Indian Ocean which are roughly spherical in shape, and consist of concentric laminae of chambers growing round a central foreign body.

Like the spherical nodules of Lithothamnion, Gypsina can be subject to the rolling action of the sea and survive.

At the time that the discussion on the systematic position of the Stromatoporoids took place little was known of the existence at the present day of large encrusting Foraminifera which could be compared with the reef-building Stromatoporoids of Palaeozoic times.

It is now known that Foraminifera of this kind are very abundant in some localities and may play an important part in the construction of modern coral reefs.

Möbius (1880, p. 84) said that Carpenteria rhaphidodendron is so abundant on the coral reefs of Fouquets Island near Mauritius that it should be considered to be one of the reef-building animals.

Crossland has sent home from the Marquesas a bottle full of large specimens of the same species, from which may be inferred that it is as important in these islands of the Pacific as it is off Mauritius. He also says in his Notes on the reefs of Tahiti (1928, p. 726), referring to Carpenteria and Gypsina, 'These large Foraminifera have evidently a great protective role and must make a distinct, if small, addition to the total mass.'

Masses of a calcareous structure were discovered by Herdman off the coast of Ceylon, and were named Ramulina herdmani by Dakin (1906). It forms 'a substantial marine deposit which cannot be of small importance in the building up of the ocean floor' (see p. 472).

The same Foraminifer occurs 'in large colonies' in 75–94 metres off Banda (Hofker, 1930, p. 128).

Gypsina plana evidently forms an important feature of the reefs of Tahiti. In the Ellice group the same species was found in great quantities at depths of 44–200 fathoms, and according to Chapman (1901, p. 202) 'it is of great importance as a reef builder'.

As recorded in this paper (p. 456) it was found not only off Mauritius but on several banks and off other islands in the Indian Ocean. From the large specimens quite recently sent to me from Abu Fanadir it seems probable that this encrusting Foraminifer may also be found in abundance in the Red Sea.

In the description of the specimen from Tahiti I have said (p. 438) that the crust reaches a thickness of 1·15 mm., but it is much thicker than that in specimens from other localities. In one from Mauritius there is a crust 5 mm. in thickness, and in another where several crusts lie one over another separated by thin layers of detritus the thickness of the mass is 20 mm. or more.

There can be little doubt that in the old controversy, Dawson, Carpenter, and others who were supporting the view that Eozoon and the Stromatoporoids were Foraminifera, had to face the objection that no large encrusting or rock-forming Foraminifera were known to exist in recent times. Carpenter (1876, p. 422), for instance, in referring to Möbius's discovery of large masses of Carpenteria rhaphidodendron at Mauritius, justifies 'his refusal to be bound down by our present very limited knowledge as to the possibilities of Foraminifera'. Our present-day knowledge of the possibilities of Foraminifera as indicated in this section would have given him a much stronger and more convincing case.

A reconsideration of the systematic position of the fossils dealt with in this paper is a matter of no little importance because if the current view is maintained that they were Hydrozoa then it follows that the Hydrozoa were in existence in the early Palaeozoic ages.

If, on the other hand, it is agreed that they were not Hydrozoa, but were Rhizopoda allied to recent Foraminifera, then there is no clear evidence of the existence of Hydrozoa earlier than late Tertiary times.

The Foraminifera have certainly a very long history. They were abundant in Cambrian strata, and among them were several genera that have survived to the present day; they extended through Silurian times into the Jurassic and the Chalk. Foraminifera are the principal constituents of the Nummilitic limestone and very abundant in other Tertiary rocks (see Heron-Allen and Earland, 1913).

Appendix on Eozoon.

Carter (1875) had expressed his view that Eozoon was not a Foraminifer two years before he published his very brief description of Gypsina melobesoides, but it carried considerable weight in the controversy because of his reputation as an authority on the Foraminifera. Of greater importance perhaps were the papers published by King and Rowney, Hahn, and others who vigorously denied its animal origin.

Carpenter (1876, p. 422) thought that the discovery of Carpenteria rhaphidodendron showed an approach to the mode of growth of Eozoon, but when Möbiüs published his great monograph on the subject in 'Palaeontographica' (vol. v) in 1878 he strongly supported the view that Eozoon was of mineral origin. An illustrated summary of his investigations and opinions was published in 'Nature' (1879, vol. xx, p. 272) and in a later number (p. 328) the replies of Carpenter and Dawson who maintained to the end that it was a Foraminifer.

More recently Kirkpatrick (1912) has brought fresh evidence in support of Dawson's original view.

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EXPLANATION OF PLATES

PLATE 26.

Photographs by Messrs. Hills & Saunders, Cambridge.

Fig. 1.—Gypsina plana encrusting a branch of dead coral from shallow water, Rahere point, Tahiti. Nat. size.

Fig. 2.—Surface view of the specimen from Rahere point, showing the areolae surrounded by thick walls, the surface hard and smooth.

Fig. 3.—Vertical fracture of the same specimen, showing the chambers arranged in vertical rows, with about thirty in each row. 'Tinoporan' structure.

Fig. 4.—Surface view of a specimen from 15 fathoms off Papeete, rather more highly magnified than fig. 2.

Fig. 5.—Vertical fracture of a specimen from 15 fathoms off Papeete showing vertical rows of four or five chambers above, and irregular spheroidal chambers between them and the support.

Fig. 6.—This photograph was taken from the same fragment as that shown in fig. 5, but in this the chambers are all spheroidal and irregularly arranged. Almost typical 'Acervuline' structure. These two figures indicate the extraordinary variability in structure seen in most specimens.

Fig. 7.—Growing edge of a dry specimen from Papeete showing a number of horizontal moniliform tubes, connected by transverse foramina, spreading over the support.

Fig. 8.—Decalcified specimen from Rahere point, showing the protoplasm of the chambers of the upper layer in groups and, slightly out of focus, the more tubular form of the protoplasm of the lower layers.

Fig. 9.—Another preparation of a decalcified specimen from Rahere showing the difference in the arrangement of the protoplasm in the upper and lower layers.

Fig. 10.—Lower layer protoplasm in a preparation of a decalcified specimen from Papeete showing in one place a stellate arrangement.

Fig. 11.—Fragment of an old dried undecalcified specimen of Gypsina plana showing the remains of the protoplasm—like black mud—in some of the chambers, the others being empty. A deposit of black mud-like substance is often seen in the chambers of many species of Stromatoporoidea and it has an arrangement similar to that in this figure.

PLATE 27.

Copied, by permiss on, from H. A. Nicholson, "A Monograph of the British Stromatoporoids", 'Palaeont. Soc.', 1885-92.

Fig. 12.—Clathrodictyon regulare vertical section.

Fig. 13.—Actinostroma clathratum vertical section.

Fig. 14.—Clathrodictyon cellulosum with walls perforated by pores.

Fig. 15.—Stromatopora hüpschii showing zooidal tubes.

Fig. 16.—Actinostroma astroites vertical section with small 'Caunopora' tubes.

Fig. 17.—Stachyodes verticillata vertical section.

Fig. 18.—'Astrorhizae' of Stromatopora concentrica.

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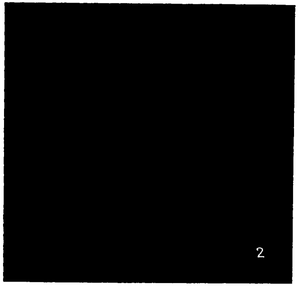
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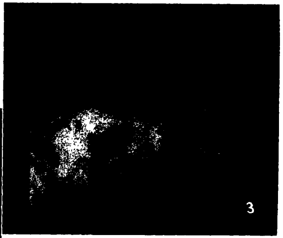
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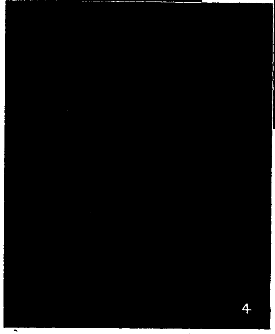
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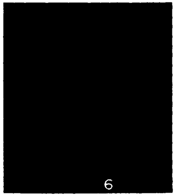
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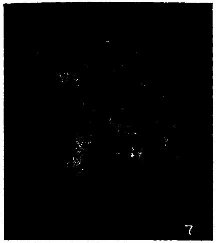
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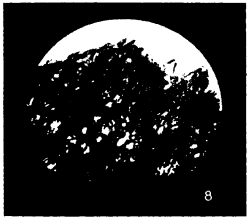
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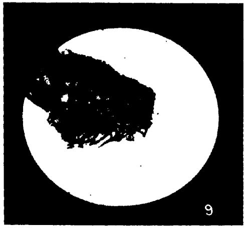
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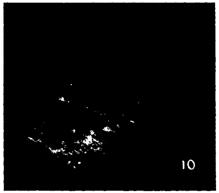
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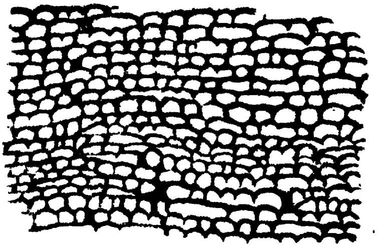
Photographs by Messrs. Hills & Saunders, Cambridge.



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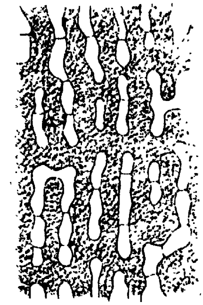

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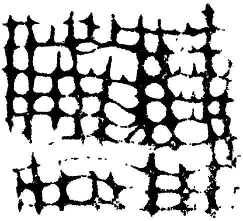

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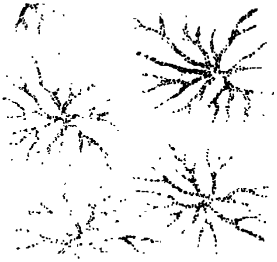

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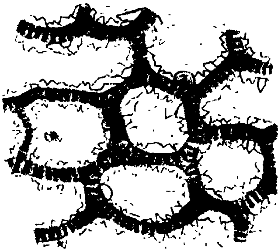

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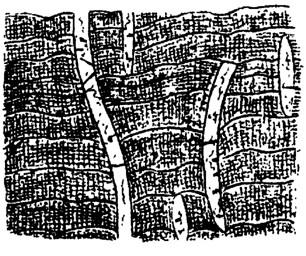

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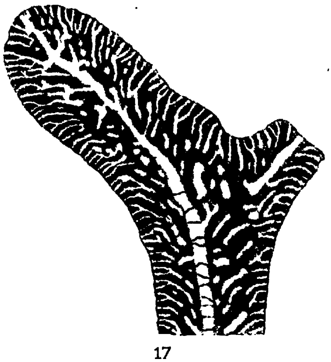

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From Nicholson, H. A., *British Stromatoporoids*

On the Teeth of the Larval Belone vulgaris, and the Attachment of Teeth in Fishes.

By

J. A. Moy-Thomas, B.A.

Demonstrator in Zoology in the University of Leeds.

With 10 Text-figures and Plate 28.

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1. INTRODUCTION.

THE larval teeth of Belone have not been previously described, but their peculiar character led Professor Garstang to put into my hands for closer study three series of sections through the heads of Belone larvae, which had been made in the previous year by one of his students. It seemed at first sight as if the teeth might be special larval organs interpolated into the life-history. A more exact knowledge of them, however, and a comparison with the larval teeth of the Eel described by Norman (1926) revealed them to be essentially similar to adult teeth, though histologically reduced in size and complexity to enable them to begin functioning at an early stage of the life-history, before attachment to the bone. Later they gradually assume ordinary tooth characters and become attached to the bone. In this free functional condition these teeth morphologically resemble placoid denticles of the adult Elasmobranchs, and it seemed an excellent starting-ground for reinvestigating the problems presented by the attachment of teeth in bony fishes. In addition to the Belone material, a series of sections of developing Salmo, Amia, Polypterus, and Lepidosiren were examined.

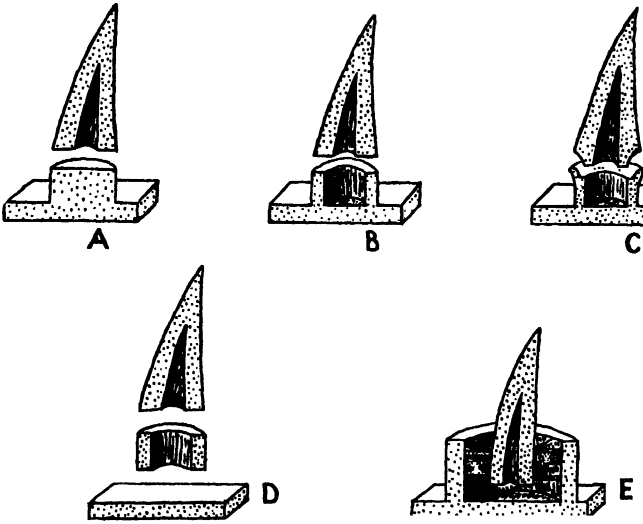
The attachment of teeth in bony fishes was first described in any detail by Tomes (1874). He confined himself to the condition in the adult, where he found there were three main methods of attachment. The teeth might be attached either to an elastic hinge (Lophius, Esox, and some Gadidae), or in a socket Saurodontidae), or by anchylosis (most Teleosts). It is this last mode of attachment by anchylosis, that has led to considerable disagreement over the homologies and origin of the tooth-bearing bones. Anchylosis was described by Tomes as taking place by an upgrowth from the tooth-bearing bone, which became attached to the base of the tooth; the extent of fusion varying in different fish. He noted that in certain cases this bone of attachment might be formed separately from the remainder of the bone. Subsequent authors, notably Hertwig (1879), Walther (1883), Klaatsch (1891), Carlsson (1894), Röse (1898), Carter (1919), and Sewertzoff (1926), have elaborated Tomes's account, and added further details of anchylosis and the bone of attachment during development. The result of these investigations showed that the fusion of teeth to the bone might take place in one of four ways, excluding the unique methods described in the Dipnoi by Semon (1901), and by Graham Kerr (1903).

1. The teeth may fuse directly to the bones, which merely become thickened in the region of attachment (Text-fig. 1 A).

2. The teeth may meet a circular upgrowth or pedestal of bone, and fuse to it. This pedestal, or bone of attachment, is hollow, and the pulp cavity of the tooth is in direct communication with its cavity (Text-fig. 1 B). The method is sometimes elaborated by the tooth base being partially enclosed in the socket of the pedestal (Text-fig. 1 C).

3. The pedestal may be formed separately from the bone as a ring like 'intermediate' bone of attachment, all three structures later becoming fused (Text-fig. 1 D).

4. The tooth may lie in a socket, formed by a circular upgrowth from the bone, from the sides of which bony trabeculae arise and anchylose with the tooth (Text-fig. 1 E).



TEXT-FIG. 1.

Diagrams of five methods of tooth attachment in fishes.

Although Tomes had been very definite in stating that the supporting pedestal was part of the bone, the later investigators held very different opinions about the homologies of this structure, considering it either part of the bone, part of the tooth, or an entirely new structure, the mode of origin observed by the individual authors determining their points of view. These divergent views opened a discussion as to the derivation of the tooth-bearing bones. If the supporting plate is part of the tooth it may be homologized with the basal plate of the placoid denticle of Elasmobranchs, in which case the basal plate gives rise to part of or sometimes the entire bone. If the supporting plate is an entirely new structure developed in association with the teeth, between them and the bone, then the tooth-bearing bones are of double origin. If, on the other hand, the supporting plate is part of the bone, and its separate origin only a precocious formation in the region of the tooth, there is no difficulty, and the tooth-bearing bones are similar to other dermal bones and are of single origin.

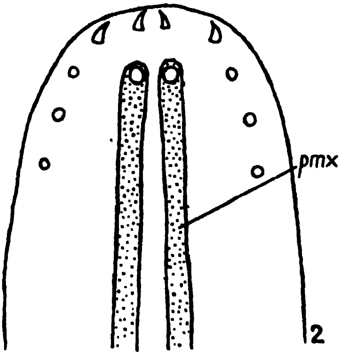
In view of the existing uncertainty of the nature of the supporting bone and the attachment of teeth in bony fishes, I decided to make a short survey of the attachment of teeth during development in fishes. For this purpose I examined sections of developing, Belone, Salmo, Amia, Polypterus, and Lepidosiren. This work was carried out in the Department of Zoology of the University of Leeds, and I should like to take this opportunity of thanking Professor W. Garstang for helpful criticism and encouragement, Professor E. S. Goodrich, F.R.S., for lending me a series of developmental stages of the Trout and Amia, Mr. J. R. Norman for allowing me to examine his sections of Leptocephali, and Professor J. Graham Kerr, F.R.S. for permission to study his Lepidosiren and Polypterus material during a visit to Glasgow.

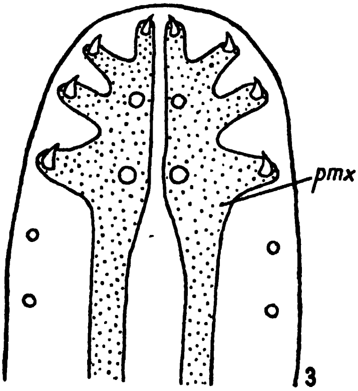
2. The Structure and Development of the Teeth of the Larval Belone vulgaris.

The material consisted of only three specimens of larval Belone vulgaris, which measured (1) 11·6 mm., (2) 15·4 mm., (3) 16·5 mm. respectively. The larval Belone is said to pass through a 'Hemiramphus' stage, the lower jaw projecting beyond the upper. This projection measured in specimen (1) 0·3 mm., in specimen (2) 0·8 mm., and in specimen (3) 1·0 mm. Specimens (1) and (3) had been cut at 6 μ, and specimen (2) at 8 μ, the celloidin-paraffin-wax method being used for embedding. All the sections were stained in iron haematoxylin and counterstained in eosin in xylol, but some were restained by Mallory's connective tissue stain.

Specimen (1) 11·6 mm.

This specimen has twelve teeth in varying states of development in the upper jaw, ranging from little more than epidermal rudiments to teeth which have pierced the epidermis. The teeth are arranged as in Text-fig. 2. The four anterior teeth occur in the extreme front end of the jaw, even in front of the premaxilla, and although they have pierced the epidermis are not attached to the bone. The teeth are minute and peg-like in appearance, and consist at this stage of a cone of solid dentine, with a very small but comparatively wide pulp cavity, containing a single odontoblast. This cone is surmounted by a cap of refractive material, presumably enamel. The teeth are about $30\,\mu$ in length (figs. 1, 2, Pl. 28). The more posterior lateral teeth show different stages of development, which is essentially the same as in any other Teleostean tooth, in which the odontoblasts have been greatly reduced. Fig. 3, Pl. 28, shows a small cone of evenly-staining dentine with but a single odontoblast at its base. In the next stage (fig. 4, Pl. 28) the apex of the dentine has become clear, and appears to have been altered into a cap of refractive enamel. From this stage the tooth advances no farther, but pierces the epidermis without more



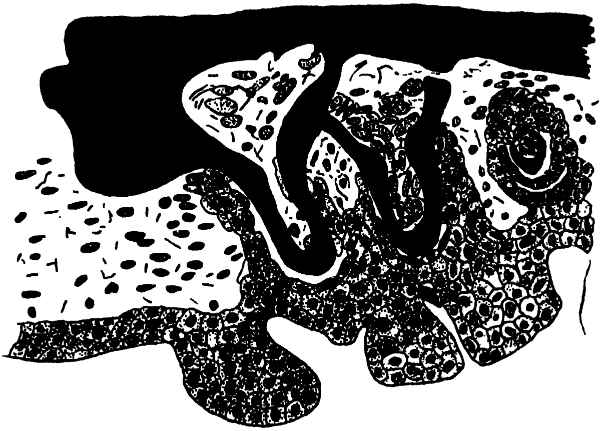


TEXT-FIG. 2. Belone vulgaris. Diagram of arrangement of the teeth in relation to the premaxilla in an 11·6 mm. specimen. (Teeth which have pierced the epidermis are represented by triangles, and teeth which have not pierced by circles.)

TEXT-FIG. 3. Belone vulgaris. Diagram of arrangement of teeth in relation to the premaxilla in a 15·4 mm. specimen.

development. The larger median teeth rudiments consist at this stage of only an enamel organ with five or six odontoblasts. They are larger than the other teeth rudiments and are being formed directly below the premaxilla.

In the lower jaw the dentaries are fairly well ossified and carry a pair of teeth placed side by side to one another on each bone, on the part protruding in front of the upper jaw. Although they are fused to the bone they do not at this stage pierce the epidermis, but are strongly recurved backwards. These teeth are considerably larger than those in the upper jaw, measuring at this stage at least 40 μ, and consist of hollow cones without the solid apex found in teeth of the upper jaw.



TEXT-FIG. 4.

Salmo fario. Section showing attachment of teeth to right premaxilla in an 18 mm. specimen.

Teeth are borne on the second pharyngobranchial, six on each side in single rows, and also ten teeth on the ceratobranchial of the fifth arch, two median, and the remainder in pairs forming an irregular triangle with the apex anterior. All these teeth are in varying stages of development from mere rudiments to teeth which have pierced the epidermis. There is practically no ossification in this region except at the base of some of the most developed teeth, where rings of supporting bone are formed (figs. 5, 6, Pl. 28). The teeth themselves are considerably larger than those in the jaws; measuring about 80 μ when fully developed. They consist of a hollow cone of dentine filled with odontoblasts and a small enamel cap. The teeth develop as hollow cones, and at no time have the solid apex characteristic of the upper jaw teeth.

Specimen (2) 15·4 mm. and Specimen (3) 16·5 mm.

Specimens (2) and (3) were found to have teeth in so nearly the same state of development and arrangement that they will be treated together. The teeth of the upper jaw are arranged as in Text-fig. 3. Two median and two lateral teeth have been added, the two median teeth being again larger than the lateral. The four most posterior median and lateral teeth have not yet pierced the epidermis. The premaxilla has grown forward and branches irregularly outwards, so that all the teeth have become attached except the four posterior median and lateral, which are very little developed. The peg-like teeth of this region, noted in specimen (1), have become enlarged and elongated at their base, increasing the size of the pulp cavity, which now contains several odontoblasts. Each anterior one is either attached or becoming attached to the premaxilla, as in figs. 7, 8, Pl. 28 (the enamel cone in fig. 8 is as deeply stained as the dentine and is almost indistinguishable). An expansion of the base of the dentine cone can be distinguished in several teeth prior to fixation.

The lower jaw teeth are eight in number, two teeth being added one behind the other in each ramus. The teeth formed in the preceding specimen have now pierced the epidermis. The new teeth can be seen becoming attached to the dentary either by direct fusion with upgrowths meeting the tooth (fig. 9, Pl. 28), or by separately developed pedestals of bone (fig. 10, Pl. 28). The sections only show the bases of the teeth owing to their backward curvature.

The pharyngobranchial teeth vary considerably in number in the two specimens and are shown most easily in tabular form:

	Left Phb. 2.	*Right Phb. 2.*	*Left Phb. 3.*	*Right Phb. 3.*	*Cerato-br.*
Specimen 2 . .	10	9	1	2	19
Specimen 3 . .	9	10	1	0	13

Each specimen has three median teeth on the ceratobranchials, the lateral teeth, however, show no very definite arrangement. The teeth are all much larger than those on the jaws, as in specimen (1). Ossification has greatly increased and the separate 'intermediate' bones of attachment have in some cases grown down towards the cartilage to form the bone, but in others the major ossification forms independently (figs. 11, 12, Pl. 28).

The teeth of the larval Belone can thus be divided into two types: (1) Those which form in the upper jaw as solid rods and pierce the epidermis before attachment, which later takes place by direct fusion, the base of the cone elongating to meet the bone. (2) Those in the lower jaw and pharyngeal regions, which develop as hollow cones and become attached to the bones either before or after piercing the epidermis. This attachment may be either direct by means of upgrowths from the bone to the tooth base, or indirect by means of independently developed 'intermediate' bony supports, which may be the first signs of ossification.

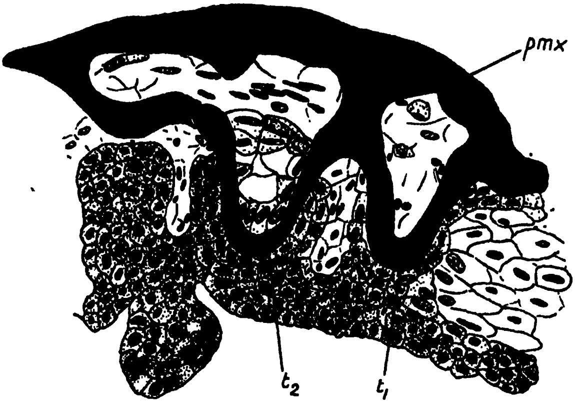
An examination of the larval teeth of Anguilla vulgaris, described by Norman (1926) with a slender apex and a comparatively large basal pulp cavity, showed a strong resemblance between these teeth and the upper jaw teeth of the larval Belone, where the solid apex is formed before the base (compare figs. 8 and 13, Pl. 28). The teeth of the Eel have no distinguishable enamel cap, and a relatively longer apex of solid dentine. It is very interesting to note that in the 5 mm. Eel the most anterior upper jaw tooth is unattached, but functional, and becomes attached in the older stages by growth at its base. The material is not very well preserved, and histological structure is to a very large extent obscure, so that the development of these teeth cannot be made out, but it is very possibly similar to that of Belone, the solid apex forming first from a very reduced number of odontoblasts.

The method of formation of enamel-like substance by the clarification of the tip of the dentine cone would seem to show that dentine may possibly be converted into enamel. If this is true it supports the view of Graham Kerr (1919, 1924) that enamel is only modified dentine.

3. The Attachment of Teeth in the Development of Fishes.

(*a*) Salmo fario.

Examination of sections of developing Trout has shown that although the teeth are met by specialized upgrowths from the bone, there is never any distinctly separate 'intermediate' bone of attachment. The first formed premaxillary teeth fuse directly to the bone, which is slightly thickened in the region of attachment, outgrowths being formed to meet the tooth base. Subsequent teeth develop and become attached in order from the outside inwards. The lateral margins of these teeth become attached to the supporting outgrowths of the preceding neighbouring teeth, or to the teeth themselves, as shown in Text-figs. 4 and 5, and the median margins to another outgrowth from the bone. The common outgrowth is not a continuous ring at its base, as can be seen from the adjacent section, Text-fig. 6, where it only joins the teeth, their two pulp cavities being in continuity. Thus here the supporting ring, although an outgrowth from the bone, is in a condition halfway between a fixed and free 'intermediate' bone. The maxillary teeth fuse directly to the bone, small outgrowths meeting them in some cases. The supports of the



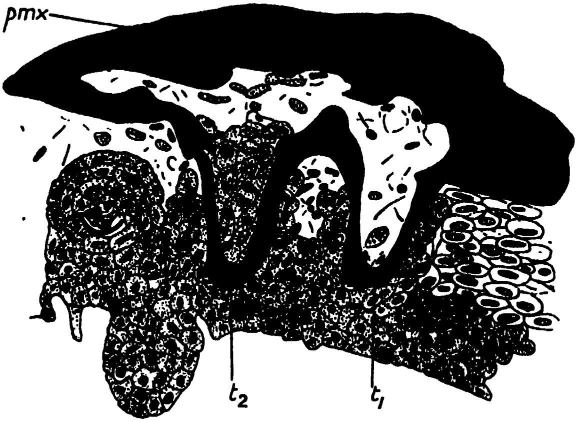
TEXT-FIG. 5.

Salmo fario. Section showing attachment of the teeth to left premaxilla in 18 mm. specimen.

palatine and vomerine teeth do not form as completely circular outgrowths, but are rather irregular.

The lower jaw teeth become attached directly to the dentary, which later becomes greatly thickened (Text-figs. 7 and 8). The ceratohyal teeth fuse on to bony pediments, which arise from the bone.

Thus it can be seen that the early teeth of the Trout become attached directly to a flat surface of bone or to bony outgrowths. The bony outgrowths are always continuous with the underlying bone and it is by their elaboration and expansion that the adult bones are formed. Although these pediments are never actually free as 'intermediate' bones, they are, as shown in the text-figures, often only very slenderly attached in early development. Later, however, the bone becomes the solid adult structure by these upgrowths meeting laterally and forming a more or less continuous mass.

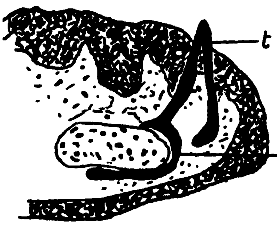


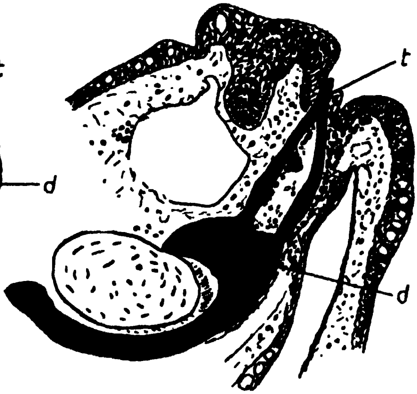
Text-fig. 6.

Salmo fario. Section showing attachment of teeth to left premaxilla in 18 mm. specimen.

(*b*) Amia calva.

The attachment of teeth in the early stages of development has already been thoroughly described by Degener (1924). Her observations and my own show that in no case is there any separate 'intermediate' supporting plate developed, the teeth always becoming attached directly to the bone, or a continuous outgrowth from it. Bone, however, tends to be developed first at the bases of, but entirely free from, the teeth. This led Degener into the view that these primary ossifications were the teeth bases homologous with the basal plate of the placoid scale, and that by their growth and expansion bone was formed. They are alternatively capable of being explained as the first signs of true bone, and give rise to the adult tooth-bearing bone. The teeth in all regions in Amia develop on these lines.





TEXT-FIG. 7. TEXT-FIG. 8.

Salmo fario. Section showing a tooth attaching to dentary in 18 mm. specimen.

Salmo fario. Section showing a tooth attached to dentary in a 30 mm. specimen.

(c) Polypterus senegalus.

Only a very limited material of Polypterus larvae in the stages of early bone development exists, but from this material it is possible to make out the development of the teeth in the anterior regions in both upper and lower jaws. The condition in Polypterus is very similar to that in Amia, and the earliest formed bone appears at the base of the teeth but distinct from them, the teeth fusing on directly. In these early stages and in later stages there is never any indication of a separate 'intermediate' ossification.

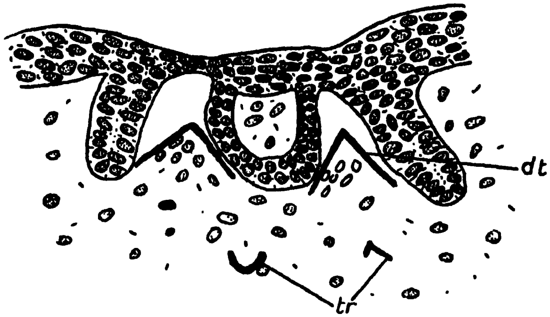
(*d*) Acipenser ruthenus.

The development of the tooth-bearing bones in relation to the teeth has been described by Sewertzoff (1926), who stresses the entirely distinct origin of tooth and bone in every tooth-bearing bone. The distinction between tooth and bone is always quite clear, the two developing relatively far apart. The distal part of the teeth becomes incorporated in the bones of the jaw. There is never any trace of a distinct 'intermediate' bone, and the development of the bones resembles that of the dermal scales of the body.

(*e*) Dipnoi (Ceratodus forsteri and Lepidosiren paradoxa).

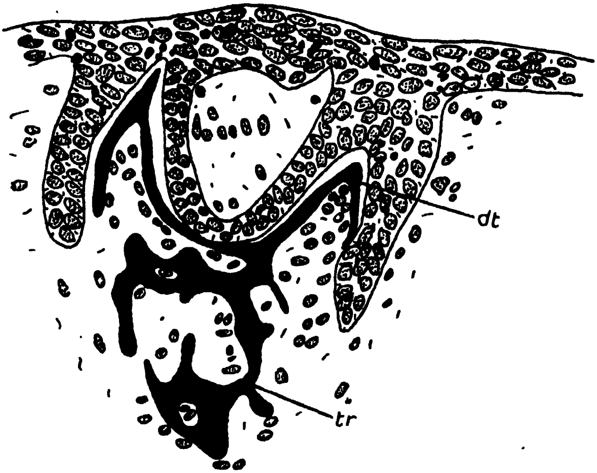
The development of the teeth of the Dipnoi has been described in Ceratodus by Semon (1901) and in Lepidosiren by Graham Kerr (1903). Semon found that the region occupied by the tooth-plates in the adult, were studded in the early larvae with separate little teeth as in other fishes. As development proceeds the bases of these dentine cones become joined together by bony trabeculae. The denticles eventually drop off and the 'tooth' of the adult consists of a mass of spongy bone.

In Lepidosiren the epidermis grows inwards into enamel organs as in normal denticles. Adjacent epidermal ingrowths come in contact (Text-fig. 9). The dentine is at first laid down as though forming separate teeth, but the bases of these teeth become continuous with their neighbours (Text-fig. 10) forming a composite cone. Below the bases of these teeth, but entirely distinct from them, small ossifications occur freely scattered in the mesenchyme, which later become attached to the bases of the teeth forming the major bulk of the adult tooth-plate. Graham Kerr concludes that these distinct ossifications are morphologically part of the tooth; but they can just as easily be interpreted as part of the tooth-bearing bones developed specially



TEXT-FIG. 9.

Lepidosiren paradoxa. Stage 34. Section showing separate origin of denticles and bony trabeculae.



TEXT-FIG. 10.

Lepidosiren paradoxa. Stage 35. Section showing fusion of denticles, and commencement of attachment of bony trabeculae to them.

in relation to the teeth, so that the so-called 'teeth' of the adult are in reality bone.

4. CONCLUSIONS.

The Chondrichthyes are entirely covered by dermal denticles, and similar denticles cover the head in the Ostracodermi, but in the latter they may have underlying skeletal dermal plates, which may form large head shields. Similarly the bony covering of the head of Osteichthyes is formed from plates of the same origin and histological structure as the body scales. In most living Osteichthyes the denticles have become confined to the mouth region on the so-called 'tooth-bearing' bones, but in a few (Siluroids, Lepidosteus, &c.) they are more generally distributed over the body. Thus it may be assumed that the origin and homologies of the body scales and dermal bones are one and the same problem, whether they arose in connexion with the sensory canals (Pehrson (1922) and others) or otherwise. The original theory of Hertwig (1874, 1879) was that teeth or denticles and underlying scales or bone are homologous with the conical apex and basal plate of the placoid denticle, and he interpreted their separate origin as a secondary modification. This view received a large amount of support, notably from Klaatsch (1891), Nickerson (1893), Röse (1898), Graham Kerr (1919, 1924), and Degener (1924).

A variation of this view was put forward by Walther (1883), and subsequently supported by Carlsson (1894) and Carter (1919), that the tooth-bearing bones are of genetically double origin, and that only that portion of the bone that supports the tooth, the 'intermediate' bone, corresponds to the basal plate of the placoid denticle.

Goodrich (1908) pointed out that these comparisons were fundamentally at fault, insisting 'that the basal plate is merely an extension of the denticle cone; there is no reason to think that it can ever develop separately. Nor is there any evidence that denticles do ever really contribute to form dermal bones. Even in the case of palatal bones of fish and amphibians, the teeth do not actually combine to build up the supporting bone, but become fused sooner or later to bony substance independently developed at their base'. These views of Goodrich have been supported by Sewertzoff (1926) in Acipenser and Polypterus.

The observations put forward in this paper make it clear that in development there is always a marked and easily recognized distinction between tooth and bone. Whatever the final state may be, either simple, or complicated as in the Dipnoi, the teeth themselves are at one time in development distinct units. The formation of the bones, however, is not from a single ossification in many cases. There is a separate 'intermediate' bone or pediment frequently developed in relation to the teeth. It has been shown that only in Teleosts and Dipnoi do these separate ossifications occur. In the Teleosts they are recorded fairly commonly, e.g. Xiphias, Pagellus, Belone, Anguilla, &c., and may even be shed with the teeth. The condition in the Trout, where, although never free, the tooth-supports are often only very slenderly attached at their bases, forms a transitional stage between fixed and free tooth-supports. In Belone they are sometimes freely developed or formed as upgrowths even on the same bone in the same individual, and all stages can be traced from one condition to the other in the same animal, viz. free ossifications, mere upgrowths, and direct fusion. This evidence is sufficient to show that separate 'intermediate' bones or pediments are only specialized portions of the normal tooth-bearing bones developed independently in connexion with the teeth.

That these separate ossifications are not primitive can be fairly safely concluded from their complete absence from such forms as Amia, Polypterus, and Acipenser. In the Dipnoi they reach their climax of specialization. Here the bony trabeculae or 'intermediate' bones are the important part of the adult tooth. Previous description of the development of the 'tooth-plates' of Lepidosiren seemed rather to suggest that the network of bony trabeculae at the bases of the original dentine cones was formed by downgrowths of these denticles, but I have from examination of the sections found no evidence to support this view, and every evidence to show that their origin is as distinct as in other bony fish.

Phylogenetic conclusions based on embryological evidence are by no means conclusive, but it is only natural to infer that the continual distinct origin of tooth and bone in development is primitive. The embryological evidence is strongly against their being part of the same structure, the placoid denticle. Particularly as the structure in question has never been observed to be formed in any other way except as a distinct unit. The main reason for supporting the Hertwig hypothesis was the close relation in development between the tooth and the first formed bone. It is true that these structures are frequently formed in close association, but it would seem an equally adequate interpretation of the facts, to suppose that the early formation of bone in these regions is actually induced by the teeth themselves. In Acipenser this association between bone and tooth is not so close as in other primitive fish, and the bone later grows to meet the teeth. Similarly in Belone the premaxilla has been shown to be formed relatively far from the teeth, but it is to the teeth that the bone grows out, forwards and laterally from the median ossification. That the presence of teeth does influence the growth of bone has been shown above to be very likely by the separate appearance of 'intermediate' bones and outgrowths formed precociously for the bases of the teeth. It has long been known that skeletal formation in Vertebrates is often directly in accordance with mechanical stresses, and it is very easy to believe that a developing tooth might be sufficient to cause bone to develop in close association with it.

Thus a review of the development of the teeth and their attachment to the bone in fishes supports the view of Goodrich that the teeth represent the entire placoid scale, and that body scales and dermal bones are new ossifications arising in the dermal tissues. These denticles of the primitive Elasmobranchs, possibly before the evolution of their own basal plates, may easily have provided a 'formative stimulus' in the development of dermal bone, the latter being formed as an alternative to a basal plate.

5. SUMMARY.

1. The upper jaw teeth of the larval Belone pierce the epidermis as solid structures, unattached to the bone and afterwards become attached by the growth of their bases directly to the premaxilla. These teeth are very similar in structure to those of the larval Eel. A curious feature is shown in their development, the original dentine cone becoming clear apparently to form enamel-like substance.

2. The lower jaw and pharyngeal teeth of the larval Belone

develop as hollow cones of dentine, and become attached to the bone either directly or by means of separate 'intermediate' pediments of bone.

3. All the teeth of Salmo, Amia, Acipenser, and Polypterus are attached to the bone directly or to upgrowths more or less continuous with the bones.

4. The bony 'intermediate' plates of the Dipnoi fuse to form the adult tooth-plate, the actual denticles disappearing.

5. The supporting pediments are shown to be only bony supports developed precociously in connexion with the teeth, and may be formed directly from the bone or separately as 'intermediate' pediments, and are in no way different from the remainder of the bone.

6. The embryology of teeth and bone in fishes suggests that they are distinct structures, and that their close association in ontogeny and phylogeny is due to the necessity of acquiring a firm base, and not because they are parts of the same structure.

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EXPLANATION OF PLATE.

LETTERING.

b, bone; *d*, dentary; *dc*, dentine cone; *dt*, denticle; *ec*, enamel cap; *eo*, enamel organ; *isp*, 'intermediate' supporting plate; *Mk*, Meckel's cartilage; *od*, odontoblast; *pc*, pharyngeal cartilage; *pmx*, premaxilla; *sp*, supporting plate of tooth; *t*, tooth; *tr*, bony trabeculae.

PLATE 28

Fig. 1.—Belone vulgaris. Upper jaw tooth of specimen 1.

Fig. 2.—Belone vulgaris. Upper jaw tooth of specimen 1.

Fig. 3.—Belone vulgaris. Developing stage of upper jaw tooth of specimen 1.

Fig. 4.—Belone vulgaris. Developing stage of upper jaw tooth of specimen 1.

Fig. 5.—Belone vulgaris. Lower pharyngeal tooth of specimen 1.

Fig. 6.—Belone vulgaris. Upper pharyngeal tooth of specimen 1.

Fig. 7.—Belone vulgaris. Upper jaw tooth of specimen 2.

Fig. 8.—Belone vulgaris. Upper jaw tooth of specimen 2.

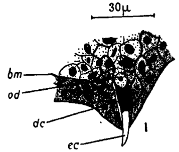
Fig. 9.—Belone vulgaris. Lower jaw tooth of specimen 3.

Fig. 10.—Belone vulgaris. Lower jaw tooth of specimen 3.

Fig. 11.—Belone vulgaris. Upper pharyngeal tooth of specimen 2.

Fig. 12.—Belone vulgaris. Upper pharyngeal tooth of specimen 2.

Fig. 13.—Anguilla vulgaris. Lower jaw tooth of 5 mm. larva.



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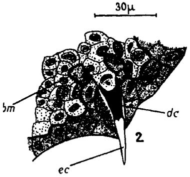

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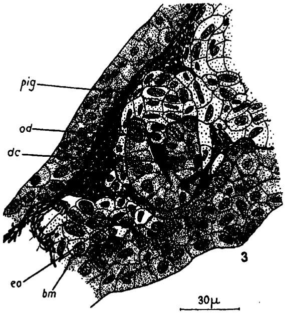

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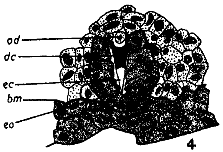

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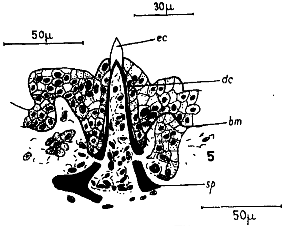

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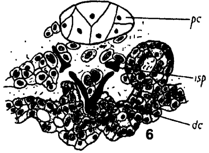

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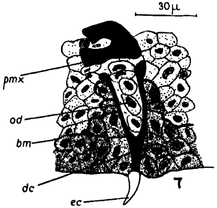

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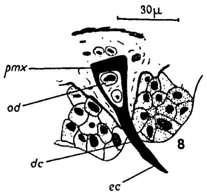

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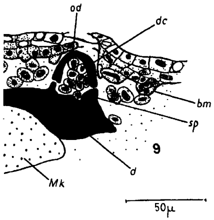

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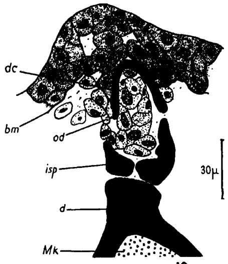

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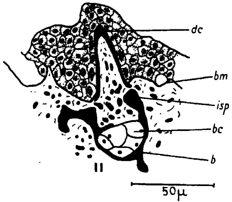

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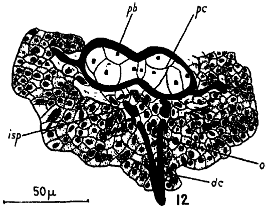

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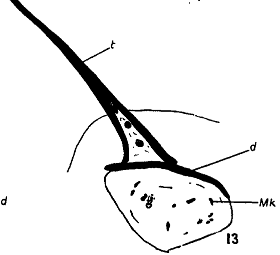

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J. A. Moy Thomas, del.

The Early Development of the Nephridia in Amphioxus: Introduction and Part I, Hatschek's Nephridium.

By

Edwin S. Goodrich, F.R.S.

With Plates 29 and 30, and 1 Text-figure.

INTRODUCTION.

It is now some thirty years since I first undertook the study of the development of the excretory organs of Amphioxus (Branchiostoma lanceolatum, Pallas). When it was discovered (Goodrich, 1902) that Amphioxus is provided with nephridia of essentially the same structure as the solenocyte-bearing protonephridia of various Polychaetes and other Invertebrates it became a matter of interest to find out how they are developed. Yet, so far as I am aware, only one author has attempted to give a detailed account of their early development in two short papers (R. Legros, 1909 and 1910). In 1909 I described the nephridia in larval stages, and since then from time to time, as opportunity offered, I have tried to make out their early development and their first origin. The investigator of this problem is confronted with many difficulties. The material is hard to obtain, difficult to preserve satisfactorily, and particularly difficult to stain well. After trying various well-known fixatives, a mixture of corrosive-acetic and picro-nitric for embryonic and Bouin's fluid for larval stages were found to give the best results. It is essential to anaesthetize the later larvae before fixation to prevent muscular contraction. Borax-carmine and picro-nigrosin, Heidenhain's iron-haematoxylin and eosin or light-green, and Mann's methyl-blue and eosin are the most satisfactory stains. The cells are so small and their derivation so difficult to make out that sections of more than about 3 or 4 μ in thickness are almost useless. The observations recorded below on embryonic stages were made on material provided by the Zoological Station at Naples for which I here express my gratitude. The larval material was preserved by me at Faro, at Naples, and in Heligoland. I have also to thank the authorities of the Biologisches Anstalt in Heligoland for sending me preserved larvae by post.

Returning now to the general problem of the homology of the excretory organs in Amphioxus already mentioned above, it may be pointed out that long ago (1895) I maintained that throughout the Invertebrata Triplobastica two distinct organs are found, of quite different origin and generally of different function, the nephridium and the coelomoduct. The latter develops centrifugally as a more or less funnel-shaped outgrowth from the wall of the coelom in higher forms provided with a well differentiated coelomic cavity; or it develops from the genital sac or 'gonad' in lower forms, such as Platyhelminths, in which the gonocoel has not yet enlarged into a coelomic 'body-cavity'. The coelomoduct, representing the original outlet for genital products, functions as a genital duct only, except when it secondarily acquires an excretory function. The nephridium, on the other hand, from the first is typically excretory, is already well formed in phylogeny before the coelomic body-cavity has appeared (Platyhelminths), originates in ontogeny from more superficial rudiments (ectodermal or ectomesodermal), and in higher forms only may come secondarily into relation with the coelom. Unfortunately nephridia and coelomoducts, which may sometimes acquire superficial resemblances to each other, were for long confused, and, indeed, still are often confused at the present day. This confusion is partly due to the fact that the nephridium itself by centripetal growth may, in some annelids, penetrate into the coelom, piercing the coelomic epithelium, and acquire a secondary opening into the coelomic cavity (Goodrich, 1895). Thus is formed from the innermost nephridial cell a nephridiostome liable to be confused, even at the present day, with the coelomostome or funnel-shaped opening of the coelomoduct. In the last century it became almost a dogma that 'nephridia' open into the coelom and are derived from its epithelium.

Coming to the Vertebrates, we find that in Craniata genital and excretory ducts are all of the coelomoduct type derived directly or indirectly from the coelomic epithelium. In spite of this they were, and still often are, taken to be nephridia. But of true nephridia no trace has yet been found for certain in any Craniate; they seem to have vanished entirely. On the contrary, in Cephalochorda, it is the nephridia that have survived and the coelomoducts that have almost if not completely disappeared. This reduction is no doubt related to the shedding by these animals of their genital products directly by dehiscence into the atrium.

How strongly observers were influenced by the prevalent confusion referred to above is shown in Boveri's account of the structure of the nephridia of Amphioxus (1892); for he described open funnels where no funnels exist.

Legros (1909 and 1910) claims to have shown that nephridia in Amphioxus develop as funnel-shaped outgrowths from the coelomic epithelium which become closed off from the coelom and acquire an opening into the atrium, or into the pharynx in the case of Hatschek's nephridium. In adopting this interpretation he also, I think, was biased by preconceived notions. My own observations do not support his view. Although I readily admit that my account given below does not solve the question of the very first origin of the nephridia, and that even the description of the early development is by no means complete, yet I think it may be claimed that it demonstrates clearly that Legros was mistaken, that the nephridium does not develop as an outgrowth from the coelomic epithelium, and that it never has at any stage an internal opening.

Therefore, in spite of the fact that the first origin of the nephridium remains undiscovered, I decided to publish my results in the hope that others more skilful or more fortunate than myself may solve this important problem.

DEVELOPMENT OF HATSCHEK'S NEPHRIDIUM.

Except for the paper by Legros (1910), referred to above, no account has been given of the early development of Hatschek's nephridium. In the previous year (Goodrich, 1909) I had described this organ in the adult Amphioxus and in the larva, and dealt with the literature on the subject. Since then the structure of the adult organ has been further considered in more detail (Goodrich, 1933), and in a paper on "Proboscis Pores in Craniate Vertebrates" (Goodrich, 1917) I referred briefly to the development of Hatschek's nephridium in a footnote as follows: 'This nephridium is developed neither from a mesoblastic funnel as described by Hatschek, nor as an outgrowth from the second somite, as described by Legros; nor, again, from the remains of the communication of the coelomic pouch with the gut, as alleged by MacBride, but from a little group of cells appearing quite early just above the mouth. Several years ago I traced these cells to a stage about thirty hours old, before the opening of the mouth; but since I am unable to find out their first origin, I refrained from publishing my results. They are the cells figured recently by Smith and Newth (fig. 4, Pl. 18), who are indeed correct in their surmise that they may represent the rudiment of the nephridium.'

Before describing the development of Hatschek's nephridium it will be well to refer briefly to its structure in the adult and in late larvae. In the adult it opens behind into the pharynx, and extends forwards along the left dorsal aorta some way beyond Hatschek's pit. It is provided along its course with short blind branches, some passing medially below the aorta and others passing dorso-laterally to the aorta (fig. 1, Pl. 29). These diverticula bear bunches of solenocytes extending into small chambers, isolated remnants of the coelomic cavity of the second somite of the larva and previously continuous with it (Goodrich, 1909, 1933).

In the metamorphosing larva of stage 5 of Willey, with open 'secondary' right gill-slits, the nephridium is a simple tube reaching to the level of the pit, and with solenocytes along its whole length. It now lies in a continuous chamber on the wall of which occasional flattened nuclei represent the coelomic epithelium (fig. 2, Pl. 29).

In a younger larva with six gill-slits of the first or future adult left series, Hatschek's nephridium is seen in transverse sections as a short blind tube lying on the ventro-lateral wall of the posterior extension of the coelomic cavity of the second somite (fig. 3, Pl. 29). Some solenocytes extend forwards, and appear in a section cutting through Hatschek's pit (fig. 4, Pl. 29).

We come now to the earlier stages obtained by the artificial fertilization of eggs at Naples. Figs. 5 A–D, Pl. 29, represent four consecutive transverse sections through a young larva (51 hrs. old[1]) in which the mouth is open but rounded, and the first gill-slit is open and functional (about stage fig. 61 of Hatschek, 1881). It is a stage older than that of the diagrammatic fig. 1, of Legros (1910) but considerably younger than that of his fig. 3. The most posterior section (fig. 5 A, Pl. 29) shows the opening of the nephridium into the gut just above the mouth. Here the nephridial cells have pushed through the endodermal wall, and an incipient lumen is seen to pass up between them and appears again in the next section (fig. 5 B). In this lumen a few cilia are already present, but it is difficult to make certain of their origin. At all events no fully formed solenocytes seem to be yet developed at this stage, and the nephridium ends blindly in the third section (fig. 5 C) in a solid mass of a few cells situated between the ectoderm and endoderm above the posterior ventral region of the second mesoblastic segment. The last section passes in front of the nephridium, cutting the second segment, which is here indistinctly divided into dorsal somite and ventral lateral plate (fig. 5 D). At this stage, then, the nephridium has acquired an opening through the endoderm and extends forwards for a short distance between the second and third mesoblastic segments.

In the next two younger stages (41 hrs. and 37 hrs.), when the mouth has just been pierced and is a small round hole, the nephridium though smaller bears much the same relations. Usually the opening through the endodermal wall of the gut has already been acquired (fig. 6, Pl. 29). Fig. 7, Pl. 29, shows this region in greater detail, and is taken from a specimen in which the opening can scarcely be said to have been formed. Even here cilia appear in the small lumen.

The next younger stage (30 hrs.) is perhaps the most important, for it is the youngest stage described by Legros and is the

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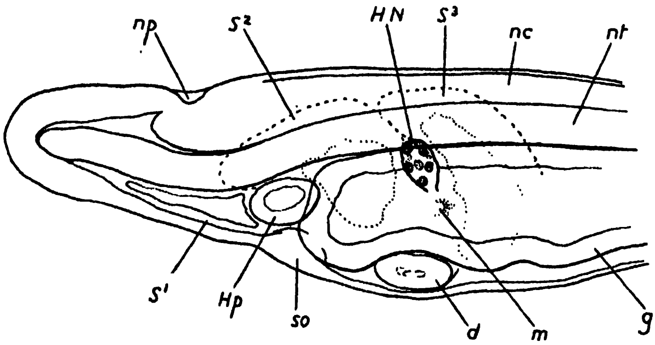
[1] As Legros (1910) remarks, the embryos of the same ages are not always at exactly the same stage of growth. But the number of hours after fertilization gives some indication of the relative state of development of the various embryos.

earliest at which the rudiment of Hatschek's nephridium can be clearly made out as such. The individual larvae, however, differ considerably in their state of development. Text-fig. 1 represents the left view of the anterior end of an advanced larva reconstructed from sections and whole preparations. The buccal diverticulum, that is to say the endodermal pouch which later opens to the exterior at the larval mouth, is present and even fused with the ectoderm; but the buccal opening is not yet pierced. The club-shaped gland is not yet tubular, nor has its duct yet opened to the exterior. The first gill-slit pouch has reached and may have fused with the ectoderm ventrally, but is still closed. The left coelomic sac of the first segment extends transversely below the notochord, but has not yet opened to the exterior to form Hatschek's pit. This is the stage of which Legros (1910) figures a diagram (his fig. 1) and two transverse sections (his figs. 2 *a* and 2 *b*). In my figure (Text-fig. 1) the point at which the mouth will be pierced is visible, and above it



TEXT-FIGURE 1.

Left side view of anterior region of a thirty-hour larva, stained and mounted whole, drawn diagrammatically to show relation of solid rudiment of Hatschek's nephridium (*HN*) to surrounding parts. Body-wall, nerve cord (*nc*), notochord (*nt*), Hatschek's pit (*Hp*), gut (*g*), and duct of club-shaped gland (*d*), in optical section. S^1 first mesoblastic segment in optical section. S^2 and S^3 second and third somites outlined in dotted line; a finer dotted line indicates coelomic cavities of first and second segments. *m*, position of endodermal buccal diverticulum and point at which mouth would later be pierced. *so*, preoral sense-organ.

is seen the rudiment of Hatschek's nephridium lodged between mesoblastic segments 2 and 3, which are indicated by dotted lines.

Three transverse sections of this stage are drawn in fig. 8 A, B, and C, Pl. 29. In the most posterior section (fig. 8 A) is seen the buccal diverticulum of the gut which has just fused with the ectoderm; the mouth, however, has not yet been pierced. The rudiment of Hatschek's nephridium is seen as a group of four cells surrounding a small lumen, in which cilia are developing, and sending between the endoderm cells of the gut a wedge-like process reaching to a point o. Here, at a later stage, would be formed the nephridiopore. The next section (fig. 8 B) shows two nuclei in the rudiment lying between mesoblastic segment 3 and 2. The most anterior section (fig. 8 C) cuts across the solid front end of the rudiment.

Fig. 9 A, B, C, Pl. 30, represents a slightly younger stage. The buccal diverticulum is less thoroughly fused with the epidermis and resembles that figured by Legros (1910, fig. 2 *b*). The most posterior section (fig. 9 A, Pl. 30), taken in front of the buccal diverticulum, cuts through the middle of the rudiment of Hatschek's nephridium. Two sections farther forward is seen its solid anterior end (fig. 9 B). The next section (not figured) shaves through its extreme tip and the hind wall of segment 2; while the next and most anterior section shows only the undivided segment 2 with its continuous coelomic cavity (fig. 9 C). Another section of a larva at about the same stage is drawn in fig. 10, Pl. 30.

A section through a stage when there is as yet no distinct buccal diverticulum, but only a slight outgrowth of a few endodermal cells, is shown in fig. 11, Pl. 30. This stage is therefore younger than the first dealt with by Legros. It may be seen that a solid rudiment is already present between mesoblastic segments 2 and 3, and has sent into the endoderm towards the point o a small process, which, however, has not yet reached the lumen of the gut.

In my batch of thirty-hour larvae, however, a few specimens can be found at a still earlier stage of development. A transverse section of one is shown in fig. 12, Pl. 30. Here the future buccal diverticulum is indicated merely by a small thickening due to the budding off of a few endoderm cells towards the surface. No distinct group of cells forming a rounded rudiment of Hatschek's nephridium can be found, but there are seen two or three cells against the wall of the gut in the appropriate place just between the posterior region of segment 2, the anterior region of segment 3, and the gut. In fig. 13, Pl. 30, representing a transverse section through a slightly earlier stage with no trace of buccal diverticulum at all, two cells flattened against the gut wall are again visible overlapped by the posterior region of the second mesoblastic segment. Although these cells can often scarcely be distinguished from the surrounding mesoblastic cells, they do not appear to belong to the wall of the second coelomic cavity, and I have little doubt that they represent the rudiment of Hatschek's nephridium.

My next batch of embryos (24 hrs.) is considerably younger. Not only do they show no trace of buccal diverticulum, but the left first mesoblastic segment is here a rounded sac, often still in connexion with the end of the gut. Yet even in these one or two cells can generally be made out between the hinder wall of mesoblastic segment 2, the anterior wall of segment 3, and the endoderm. A transverse section of such a stage is shown in fig. 14, Pl. 30. There can be little doubt that these cells represent the early rudiment of Hatschek's nephridium. They lie outside the wall of segment 2, are not derived from its epithelium; but whence they have arisen I am unable to say.

From the account given above it will be understood that my observations agree with those of Legros down to the stage with about six gill-slits of the left series, where Hatschek's nephridium has essentially the structure depicted in his diagram (1910, fig. 5 *b*) of a somewhat older larva with nine gill-slits. The nephridium is a straight canal open behind, blind in front, and with solenocytes set along nearly its whole course. Their tubes pierce the wall of the nephridial canal to reach its lumen (compare my figure drawn from the living larva, fig. 38, Pl. 15, 1909). But from such a stage back to the earliest larval stages our results differ radically. For, according to Legros, in earlier stages the nephridium is in the form of an open funnel. He begins his description of the development with the stage at which the endodermal buccal diverticulum has just met the epidermis and the mouth is not yet open (his diagram fig. 1, corresponding to my stage, figs. 9, 10, and 11, Pl. 30). The rudiment of Hatschek's nephridium is said to appear as a backwardly directed diverticulum of the 'vésicule intermédiaire', that region of the still undivided left second mesoblastic segment which lies between the myotome above and the lateral plate below. This funnel-shaped outgrowth from the posterior wall of the second segment is said to end blindly just above the buccal diverticulum. Of the existence of such a funnel open in front, I can find no evidence at this stage, nor at any earlier or later stage. On the contrary, as already explained, the rudiment of the nephridium at about this stage always has the appearance of a more or less solid group of cells in the same position as Legros's alleged funnel, but it is a separate independent structure lying between segments 2 and 3. By careful focusing one can always make out, in good and well-stained sections, that the anterior end of the rudiment is not open and is covered over by the coelomic epithelium of segment 2.

The next older stage described by Legros is that at which the buccal diverticulum has fused with the epidermis and the mouth is about to be pierced (his figs. 2 *a*, and 2 *b*, corresponding to my fig. 8, Pl. 29). The nephridial funnel is said to have already acquired an opening into the gut, so that there would now be a free passage from the coelom of segment 2 to the lumen of the gut. Legros figures a transverse section passing in front of the alleged funnel, and another five sections farther back through the pharyngeal opening. But no detailed figure is given at this, or indeed at any stage, of a section through the funnel itself.

Legros appears to have studied only transverse sections which are not really suitable for determining whether the nephridial rudiment has an anterior opening or not. To confirm my own view that such an opening is not present I have examined longitudinal sections, and, though it is difficult to obtain satisfactory sections in exactly the right plane, such can occasionally be found. Figs. 15 and 16, Pl. 30, represent longitudinal sections of two larvae at the stage when the mouth is about to be pierced. They pass dorsally to the buccal diverticulum, and show clearly enough the rudiment of Hatschek's nephridium as a little independent group of cells lodged between the second and third mesoblastic segments, and without any opening into the coelom of the second segment.

The solenocytes themselves, according to Legros, develop from the coelomic epithelium lining the inner wall of his 'vésicule intermédiaire'. He supposes that the tubes grow outwards and backwards, and penetrate into the elongating funnel. Legros gives a diagram purporting to show the structure of the nephridium at this stage (his fig. 4 *c*, of a five-day larva). The 'vésicle intermédiaire' is now supposed to become longitudinally divided into an inner chamber containing the solenocytes and an outer canal formed by the original funnel and its forward extension derived from the outer wall. At the same time the free ends of the tubes are supposed to become enclosed as the funnel closes, and so come to pierce the wall of the completed organ.

My own observations yield no evidence whatever of this supposed origin of the solenocytes separate from the canal. On the contrary, so far as I can make out, they are always derived from the nephridium itself, and simply grow out from it as the canal lengthens. But the exact steps in the process I am not yet able to describe in detail in the case of Hatschek's nephridium. It is clear that solenocytes can only function if their tubes are fixed in and pierce the wall of the canal. Legros's account of the formation of solenocytes with tubes and flagella complete, and of their secondary connexion with the nephridial canal, seems to me quite unintelligible.

A summary of the results recorded in this Part I will appear shortly in Part II, now in preparation dealing with the development of the paired nephridia.

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EXPLANATION OF PLATES 29 and 30.

LETTERING.

a, artifact space; *bd*, endodermal buccal diverticulum; *cnu*, nucleus of coelomic epithelium cell; *cr*, cells probably representing very early rudiment of Hatschek's nephridium; *cs*, coelom of mesoblastic segment; *d*, duct of club-shaped gland; *end*, endostyle; *ep*, epidermis; *g*, endodermal wall of gut; *gr*, groove from preoral pit; *Hn*, Hatschek's nephridium; *Hp*, Hatschek's pit; *lao*, left dorsal aorta; *mo*, mouth; *mp*, point at which mouth will open; *nc*, nerve cord; *nt*, notochord; *o*, opening into gut of Hatschek's nephridium; *prm*, preoral muscle; *pop*, preoral pit; S^2 and S^3, second and third mesoblastic segments or somites; *so*, sense organ; *sol*, solenocyte.

The magnification of figs. 1, 3, 4, 5 A, and 6 is given on the scale attached to fig. 3; and that of figs. 5 B, C, D, and 7–16 is given on the scale attached to fig. 12.

PLATE 29.

Fig. 1.—Transverse section of Hatschek's nephridium in adult, showing short dorsal and inner branches.

Fig. 2.—Transverse section of Hatschek's nephridium in a late larva from Naples with open right slits. Stage 5 of Willey.

Fig. 3.—Transverse section of Hatschek's nephridium of a younger larva from Faro, with rudiments of six right slits, passing between mouth and preoral pit.

Fig. 4.—Transverse section of same larva passing farther forward beyond nephridial canal.

Fig. 5 A–D.—Four transverse sections of fifty-one-hour larva. A, most posterior, cuts through opening of Hatschek's nephridium. B, second section farther forward, shows small lumen in nephridium. C, next section, cuts through its solid anterior end. Next but one section, D, shows coelomic cavity of second mesoblastic segment.

Fig. 6.—Transverse section of thirty-seven-hour larva cutting through opening of Hatschek's nephridium.

Fig. 7.—Part of transverse section of thirty-seven-hour larva, slightly younger, in which opening of Hatschek's nephridium has not yet been formed.

Fig. 8 A, B, C.—Consecutive transverse sections of thirty-hour larva. In most posterior section A are seen buccal diverticulum fused with ectoderm, and above it rudiment of Hatschek's nephridium acquiring opening into lumen of gut. In B nephridium is between segments 2 and 3. Most anterior section C cuts through solid end of nephridial rudiment.

PLATE 30.

Fig. 9 A, B, C.—Transverse sections of thirty-hour larva with buccal diverticulum just fused with ectoderm. A shows solid rudiment of Hatschek's nephridium between segments 2 and 3. B, two sections farther forward, cuts solid end of nephridial rudiment. Next and most anterior section, C, cuts wall and cavity of segment 2.

Fig. 10.—Transverse section of thirty-hour larva similar to that drawn in fig. 9 A and of about same stage.

Fig. 11.—Transverse section of thirty-hour larva slightly younger, showing process from solid rudiment of Hatschek's nephridium growing between endodermal cells towards lumen of gut.

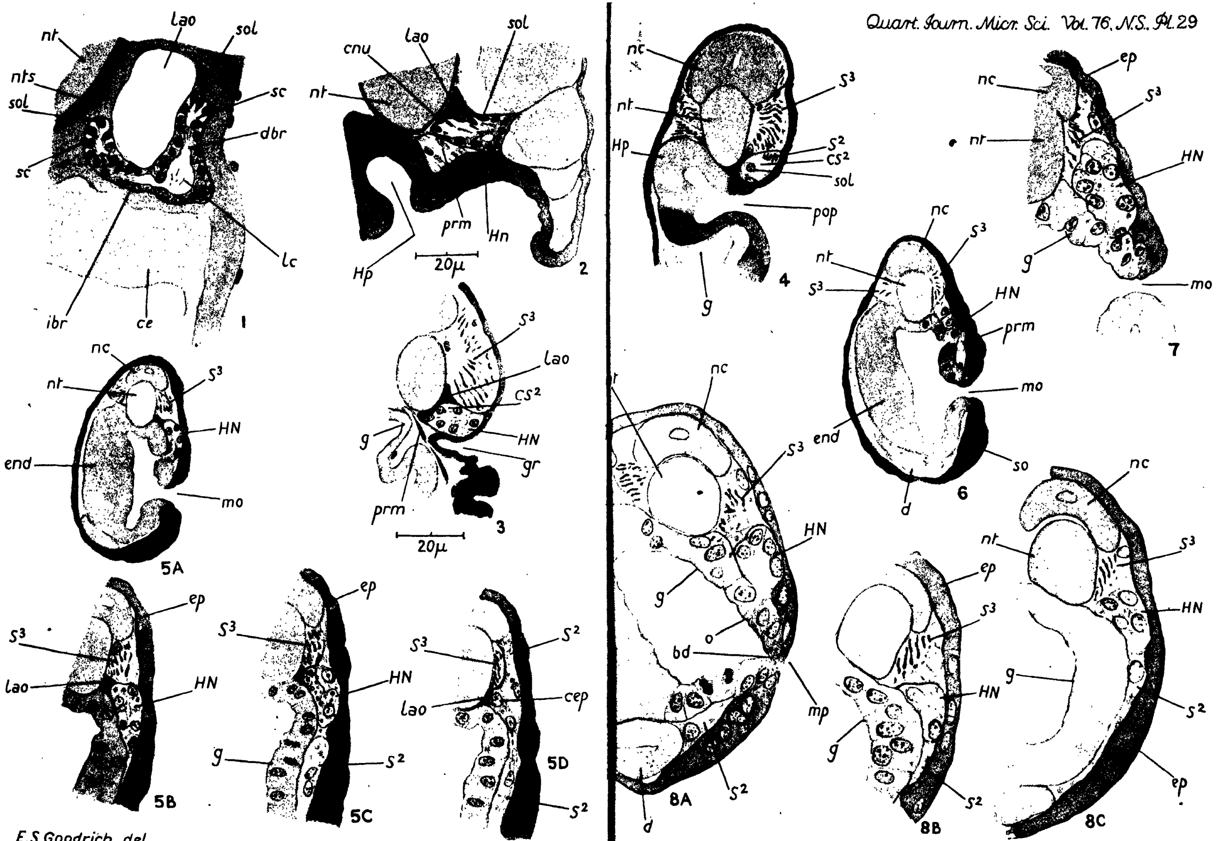
Fig. 12.—Transverse section of younger thirty-hour larva in which small buccal diverticulum has not yet fused with ectoderm. Between segment 2 and endoderm two cells are seen which appear to represent rudiment of Hatschek's nephridium.

Fig. 13.—Transverse section of still younger thirty-hour larva with no buccal diverticulum. Two cells in same position as those of fig. 12 appear to represent rudiment of Hatschek's nephridium.

Fig. 14.—Transverse section of twenty-four-hour larva at stage with no trace of buccal diverticulum, and first left mesoblastic segment a round sac still in connexion with anterior end of gut. Two cells, apparently rudiment of Hatschek's nephridium, are seen between gut and segment 2.

Fig. 15.—Part of longitudinal section of thirty-hour larva showing rudiment of Hatschek's nephridium as solid mass of few cells between segments 2 and 3. An arrow points to position of buccal diverticulum in a more ventral section.

Fig. 16.—Part of longitudinal section of another thirty-hour larva showing similar rudiment.



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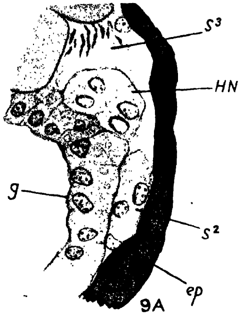

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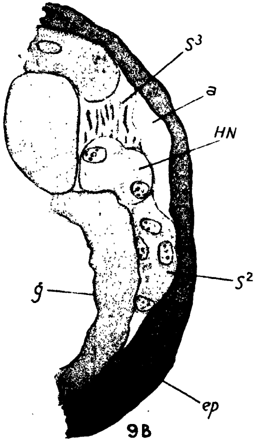

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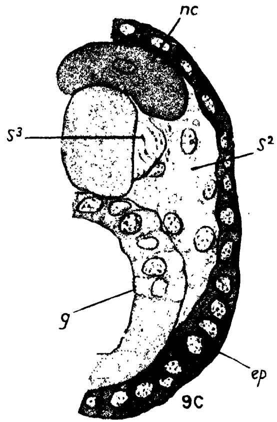

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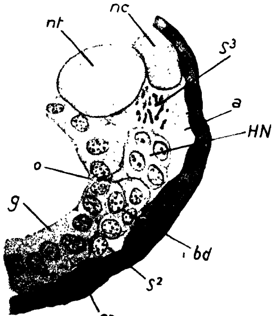

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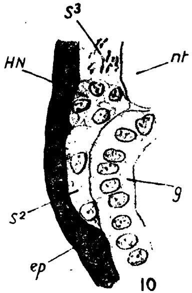

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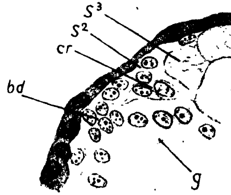

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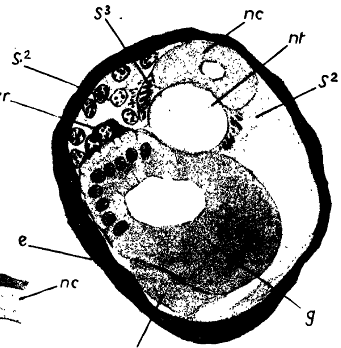

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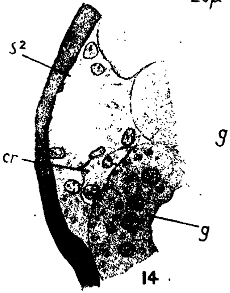

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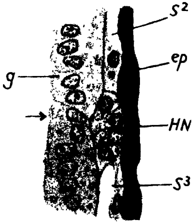

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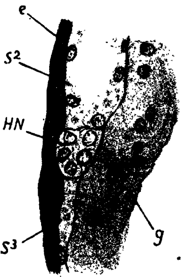

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E.S. Goodrich del.

The Innervation of the Heart of Crustacea.
II. Stomatopoda.

By

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Lwów (Poland).

With Plate 31 and 13 Text-figures.

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INTRODUCTORY.

WHEN working out the innervation of the heart of the Decapod Crustacea—the subject of my previous publication[1]—I met some difficulties in making the preparations and in the interpretation of the observed elements. I then turned to the Stomatopods expecting to find there some enlightenment on several dubious questions, since the size and the shape of the heart in these animals as well as the position of the main nerve-trunk in this organ allowed one to hope that the investigations would here be easier than in the Decapods. Though for various reasons the nerve-system of the heart in the Stomatopods, too, could not be thoroughly analysed, and I am not fully satisfied with the obtained results, I proceed to give an account of my researches.

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[1] This Journal, vol. lxxv, Part II, 1932.

HISTORICAL.

Respecting the bibliography of the subject we may say that few writers have paid attention to the heart-nerves of Stomatopods. In 1883 C. Claus, when describing the circulatory system in the larval form of these animals, noticed large nerve-cells lying on the heart-tube at regular intervals, each behind an ostium. More precise observations are due to J. Nusbaum who recorded them in 1899 in two papers, one published in German and the other in Polish, the latter somewhat more detailed and containing more figures. This writer described various fibres in the main trunk of the heart of Squilla mantis and added some remarks about the nerve-cells.

The same nervous trunk has been also seen and figured by Police (1909).

MATERIAL AND METHODS.

During my sojourn in Naples in 1930, thanks to the amiability of Professor Dr. R. Dohrn, Director of the Zoological Station, I was provided with a sufficient quantity of large specimens of Squilla mantis, and could begin my investigations. The methods described in my previous paper, i.e. staining with methylene blue and rongalit white, were chiefly used. The staining of the nervous elements in the heart was obtained by submerging the tissue in a solution of the dye, or by injecting it into the body of the living animal. The injection was made in the abdomen from its ventral or dorsal side; in the latter case the needle was introduced under the carapace between two segments, and the dye pressed into the pericardial cavity. One must be on one's guard during these manipulations, as the animals use not only their claws but also the spines of the telson as weapons of defence. One to six hours after the injection the dorsal part of the carapace with the adjacent muscles was removed and the heart exposed. Its main nerve-trunk, lying superficially on the dorsal wall, could be observed without sectioning the heart and even without taking it out from the body. Thus the heart can be left in situ till the staining succeeds.

In order to avoid shrinking of the heart-wall I employed a proceeding which had proved useful when making preparations of the digestive tube of small animals, i.e. I introduced glass tubes of adequate calibre in the organ before fixation, and in this way the heart remained stretched in the fixing and washing fluids.

Besides the vital staining, I also employed the staining of the nerve-tissue by means of silver salts, viz. the method of Cajal in the modification of Schultze and that of Bielschowsky modified by Gross. The description of these methods as they had been used by me is to be found in my previous papers about Cephalopods ('Arch. de Zool. exp. et gén.' 66, 1927). The results obtained with Squilla are not bad, but in many preparations the impregnation of the connective tissue hinders the observation of nerve elements.

DESCRIPTIVE.

I. SOME DATA CONCERNING THE ANATOMY OF THE HEART IN SQUILLA MANTIS.

The circulatory system of the Stomatopoda had been described and figured in the works of Milne-Edwards (1834), Claus (1883), and Giesbrecht (1913). The figure showing the heart and the vessels in Squilla mantis given by Milne-Edwards is not quite exact. The representation adopted by Giesbrecht in his work about the anatomy of the Crustacea is based on the figure of Claus. As this writer had investigated the larval form I think it not superfluous to give a view of the heart in an adult specimen of Squilla mantis—the object of my investigations.

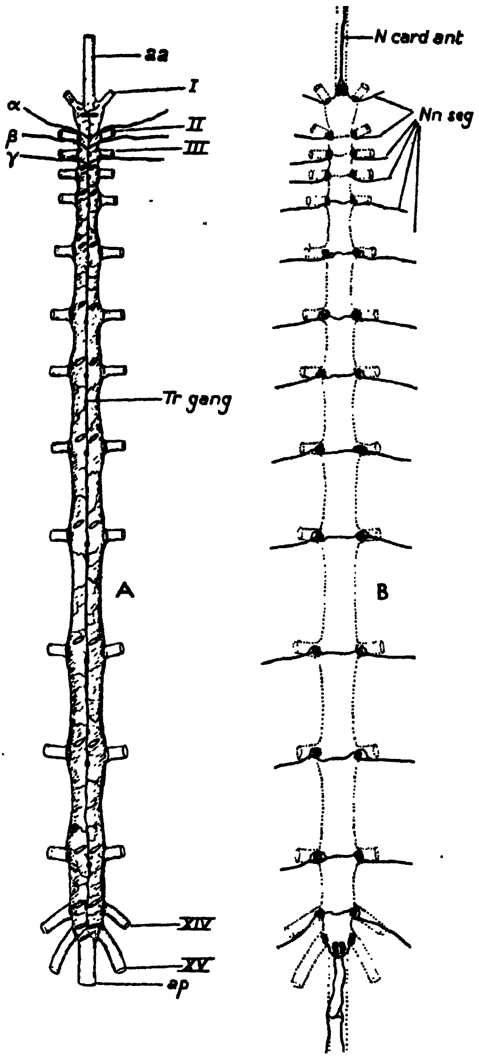
Text-fig. 1 shows the heart of Squilla, with all the arteries which arise from it. We see that it has the shape of a long tube of unequal diameter. The anterior part presents a dilatation which is followed by a narrower part. In the next segments the heart-tube becomes wider again but its width diminishes towards the posterior end.

The length of the heart-tube is approximately equal to half the length of the body; thus in an animal 17 cm. long it was precisely 8·5 cm.

Thirty-two arteries arise from the heart. Two of them are the prolongation of the heart-tube in the median line, the others are paired vessels running to the sides. The first pair has an antero-lateral direction, the two most posterior pairs, XIV and XV, run outwards and backwards; the remaining twelve pairs (II to XIII) originate from the heart at a right- or slightly oblique angle. In order to simplify the description we shall use the term anterior and posterior aorta for the two median vessels, and we shall indicate by the numbers I to XV the lateral arteries.[1] Text-fig. 1 shows that the exit-points of these vessels are not regularly situated. The first pair of lateral arteries branch off near the anterior aorta and at a certain distance from the second pair. The exit-points of the subsequent five pairs II–VI lie nearer, those of the following pairs VII–XIII farther from one another. The last three pairs are again situated close to each other, especially XIV and XV; the last pair branch off quite near to the posterior aorta.

In the figure given by Milne-Edwards in his 'Histoire Naturelle des Crustacés' sixteen lateral vessels are represented. It seems that this writer was not aware that the two anterior thoracic arteries have a common trunk arising from the heart (our lateral artery II), and he has drawn by mistake both branches of this trunk as originating from the heart.

[1] The nomenclature used by Giesbrecht is as follows:
Aorta anterior is called aorta.
Aorta posterior is called arteria dorsalis pleica.
Lateral arteries are called:
pair I, arteriae laterales cephalicae.
pairs II–VIII, arteriae thoracicae.
pairs IX–XV, arteriae pleicae.



TEXT-FIG. 1.

A. General view of the heart of Squilla mantis from the dorsal side. *aa, ap,* anterior and posterior aorta; *Tr gang,* ganglionic trunk; α, β, γ, dorsal nerves; I, II, III, XIV, XV, lateral arteries. B. Nervous system of the arterial valves. The heart is seen from the ventral side. *N card ant,* Nervus cardiacus anterior; *Nn seg,* Nervi segmentales cordis.

All the arteries possess at their origin valves supplied with muscle-fibres.

On the dorsal wall of the heart the ostial orifices are noticeable. Their shape and dimensions can well be seen on the microphotograph in Text-fig. 2. The first pair of ostia is situated between the first and the second pair of arteries (Text-figs. 1 A, and 4). According to the representation of Claus and Giesbrecht these ostia lie in the larval form just before the second lateral arteries. If the figure of Claus be correct, the position of these ostia in the larva would differ from their position in the adult form, as in the latter they lie nearer to the anterior end.

The second pair of ostia lies in the vicinity of the fourth pair of arteries, the third near to the fifth, and so on, each successive ostium being situated in the neighbourhood of each pair of arteries. In consequence, the last two pairs of ostial orifices lie near to each other. There are in all thirteen pairs of ostia. The existence of the last pair was denied by Claus, but they are surely present.

The heart-tube is made up of muscle-fibres which are arranged in two layers. The inner layer, much thicker, consists of fibres which turn in right-handed spirals. The muscle-bundles are interconnected by anastomosing muscle-fibres which branch off at very small angles, and are therefore better seen when the heart-wall is distended. Upon this muscle-layer lies outwardly a second layer of fibres of smaller calibre which are arranged in a much looser and more irregular network; the meshes of this network are elongated in an opposite direction to that of the inner layer, i.e. they turn in left-handed spirals. The two layers are not independent from each other, as branches from the thicker inner layer of fibres deviate outwards to enter the superficial plexus. In the anterior and posterior parts of the heart and in the vicinity of the ostia the arrangement of the fibres is more complicated.

The muscular heart-tube is surrounded by a coat of connective tissue. Several bundles of this tissue expanding from the dorsal side of the heart may be considered as the suspensory ligaments of the heart.

II. General Arrangement of the Nerves supplying the Heart.

1. Three Systems of Nervous Elements.

As was mentioned, Claus and Nusbaum have stated that the heart of the Stomatopoda is accompanied by a nervous trunk containing ganglionic cells. I have confirmed the discovery of these writers during my investigations and, moreover, I could distinguish in the heart of Squilla three systems of nervous elements homologous with those which have been described by me in Decapods, viz.:

(1) A local nervous system;

(2) A system of regulator nerves connecting the heart with the central nervous system (Nervi cardiaci dorsales);

(3) A system of the nerves of the arterial valves.

The local system is made up of neurons, the cell-bodies of which lie in the nerve-trunk running alongside the dorsal wall of the heart (Text-figs. 1 A, 2, and 3). The topography of this ganglionic trunk in Stomatopods is remarkable for the fact that it lies on the outside of the muscular fibres in the layer of the connective tissue enveloping the heart-tube, whereas in the Decapoda this trunk is situated on the inner surface of the muscular tube, or, when partly covered with muscle-fibres, very near to this surface. In Isopods I have found the ganglionic trunk on the inner side of the myocardium. Thus the Stomatopods differ in this point from the two investigated groups of Crustaceans, and, on the other hand, they have this superficial position of the main trunk in common with the Xiphosura and with the Scorpionidae.[1]

The system of the regulator nerves consists of three pairs of nerves—Nervi cardiaci dorsales—which run from the central nervous system and join the ganglionic trunk approaching the heart from its dorsal side. Text-figs. 1 A, 4 A, α, β, γ.

The third system—the nerves of the arterial valves

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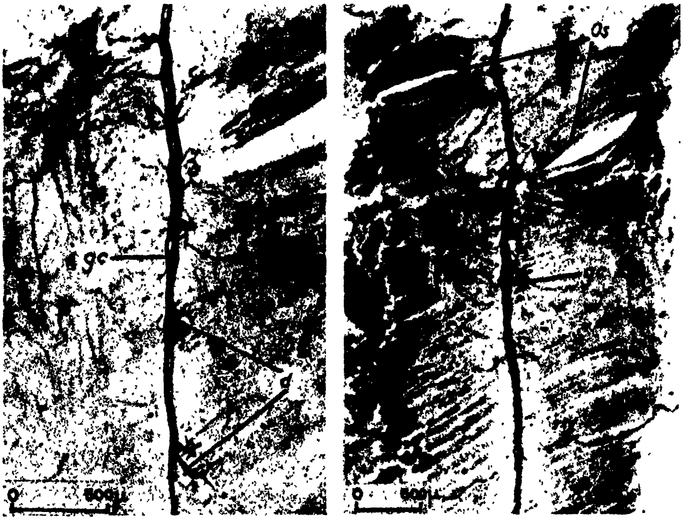
[1] I have investigated the nerves of the heart in Euscorpius carpathicus, and intend to give a description in the future.

—is made up of fibres which, being quite independent from the local system and from the regulatory nerves, take their course directly to the valve-muscles of all the arteries arising from the heart, Text-fig. 1 B. We shall use the term Nervus cardiacus anterior (*N card ant*) for the nerve supplying the valve of the anterior aorta, and Nn. cardiaci segmentales (*Nn seg*) for the nerves going to the remaining valves.



TEXT-FIG. 2.

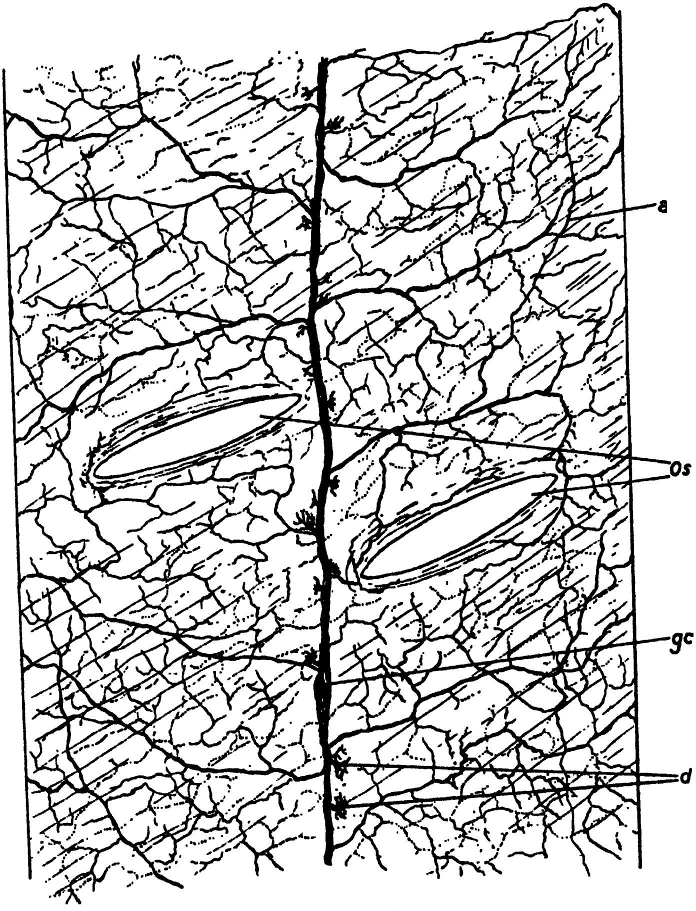
Microphotographs showing the main nervous trunk (ganglionic trunk) of the heart. *gc*, ganglion cell; *d*, dendrites; *Os*, ostia.

2. General course of the Nerve-fibres in the Heart-wall.

The main nervous trunk gives off branches of unequal length and character (Text-fig. 3):

(*a*) Short branches of stout calibre, and richly ramified, lie in the neighbourhood of the ganglionic trunk. We shall return to them when speaking about the dendrites of the ganglion cells (Text-figs. 2 A and 3, *d*.)

(*b*) Long branches arise from the ganglionic trunk at various angles and, while passing on the lateral and ventral wall of the heart, give off numerous dividing branches which



TEXT-FIG. 3.

Nerves of the dorsal wall of the heart. *gc*, ganglion cell; *d*, dendrites; *a*, fibre ending in the territory of another branch; *Os*, Ostia.

penetrate the heart-wall in its whole thickness. One part runs deeper and distributes its terminal fibres between the inner layer of the muscles; the other part takes a more superficial course and divides into thin branches in the connective tissue enveloping the myocardium, and thence sends fibres to the outer layer of muscles. Thus, all the muscle-fibres of the heart, those of the arterial valves excepted, are supplied with nerves from the ganglionic trunk. Whether or not the nerves also terminate in the connective tissue is difficult to ascertain, as incomplete staining can give rise to such images when a nerve-fibre does not reach the muscles and stops in the connective tissue.

When tracing the long branches we often find the areas of distribution of one branch overlapping the territory of neighbouring branches (Text-fig. 3, *a*); but the anastomotic junctions which are of common appearance in the Decapods are rare in Stomatopods and seem to present accidental contacts of two fibres.

As a regular feature of the long branches arising from the ganglionic trunk we must consider their being made up of fibres of unequal calibre. At many places there are two fibres running parallel to each other both giving off dividing branches. However, the two nerves cannot be traced up to the finer branches, and one does not meet with regular pictures of double innervation such as may be observed in the skeletic muscles of various Arthropods. On the other hand more than two fibres, sometimes three or four, may enter a nerve branching off from the median trunk. Some data respecting the origin of these fibres will be discussed further.

It will be noted that the majority of fibres making up the main trunk are nothing else than the axons of the ganglionic cells of the local system. The branches described above are the ramifications of these axons.

With regard to the endings of the nerves on the muscles, I must repeat my previous statements about the absence of any special end-organs. In the methylene-blue preparations the nerves end, after many sub-divisions, as thin filaments which for the most part break up in small beads.

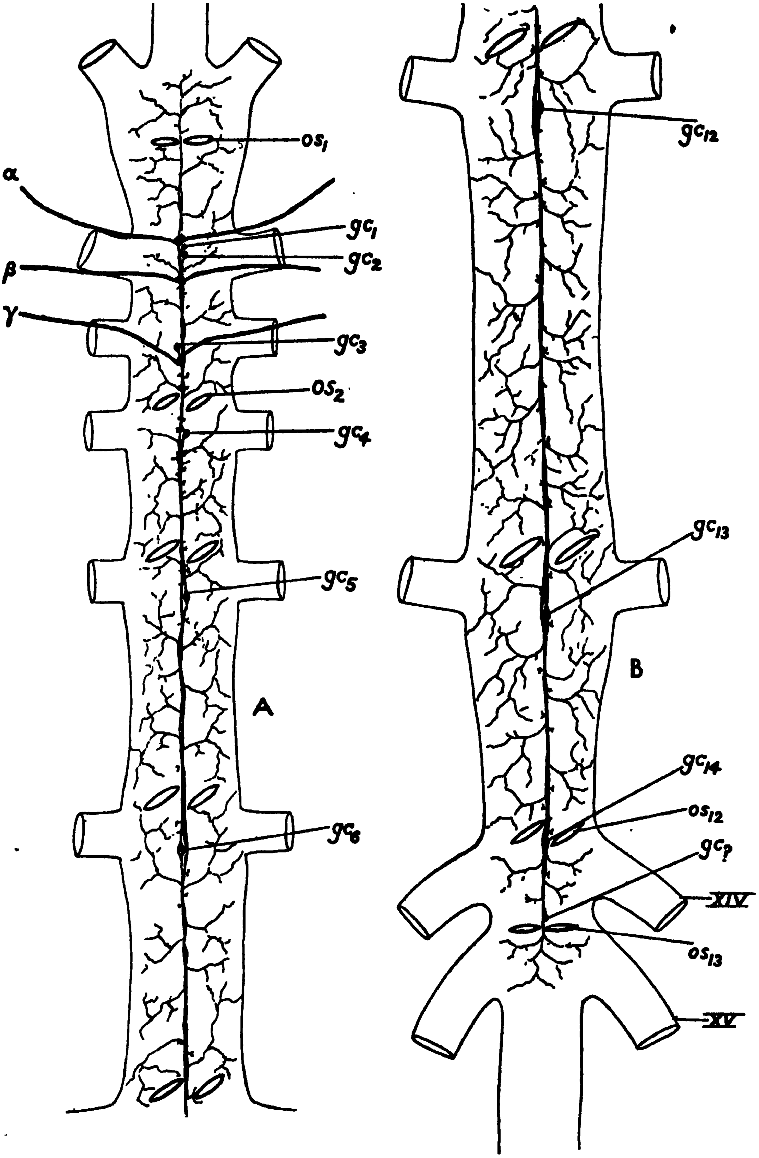
III. LOCAL SYSTEM.

Ganglion cells.—According to the observations of Claus the nerve-cells are lying in the median trunk one in each segment behind the ostial orifices; it should therefore be easy to ascertain their total number. As a matter of fact, this position of the cells, indicated by Claus, can be found in the majority of segments; but difficulties arise when we turn to the anterior and posterior parts of the heart. As we have seen, the situation of the ostia is here different and, moreover, nerve-cells are present where no ostia exist. Besides, the cells in the anterior part of the heart are smaller than in the farther segments and their staining is less satisfactory.

The Text-figs. 4 A and 4 B show the situation of the cells in the anterior and posterior part of the heart. In A the first two cells, gc_1, gc_2, lie between the second pair of arteries, or a little posteriorly between the second and the third pair. The first of these cells, which is the smallest of all, can rarely be distinguished as it oftentimes has the aspect of an ovoid body which might be regarded as a swelling of the nerve. On the other hand, in some preparations two similar bodies can be seen, and the supposition as to the presence of another cell of equally small size might be found justifiable. However, as the cellular character of this second swelling could not be demonstrated I have omitted it in the drawing. The second ganglion cell lies near to the first one, the third, gc_3, between the third pair of arteries. In the following segments the ganglion cells can easily be found one behind each pair of the ostia (Text-fig. 4 A, gc_{1-6}; 4 B, gc_{12-14}). The cell gc_{14}, corresponding to the fourteenth pair of arteries, lies often rather between the ostial orifices than behind them. This is the last cell whose presence is beyond any doubt. In some cases I saw what might be regarded as a small cell between the last (thirteenth) pair of ostia (Text-fig. 4 B, $gc)_?$. As in this part of the heart leaf-like or cell-like masses often stain with methylene blue, I am compelled to express some doubts about the real existence of this cell.

Putting all these observations together, we assume that the number of the nerve-cells is most probably fourteen and certainly not greater than sixteen.

The size of the cells varies in different parts of the heart. The first cell, the smallest, is ca 30μ; the following ones, i.e. the second, third, and fourth, are larger, 60 to 80μ; the fifth, which is at the same time more elongated than the foregoing, is



TEXT-FIG. 4.

A, anterior, and B, posterior, part of the heart of Squilla mantis. Note the position of the ostia os_1, os_2, os_{12}, os_{13}, and of the ganglion cells gc_{1-3}, &c. The presence of the fifteenth cell $gc_?$ is doubtful; α, β, γ, dorsal nerves.

ca 140μ. Then follow the largest cells of all, up to 180μ, but the last ones are again a little smaller.

The size of the cells varies obviously with the size of the specimens. All the given numbers have been obtained by measuring the cells of the same heart. In the figs. 1, 2, 3, Pl. 31, the first, second, fifth, and eighth cell drawn from the same preparation are represented.

The definition of the shape of the cells offers some difficulties. At a superficial observation all the cells, the four anterior excepted, look bipolar, with two stout processes arising from the opposite poles and running in the ganglionic trunk, but on more careful examination one can find some cells sending off, besides the two named processes, one or two additional arborescent outgrowths (figs. 4 and 5, Pl. 31). The general structure of the cells, however, their symmetrical situation, the behaviour of their long processes, do not give sufficient ground to distinguish various kinds of cells endowed with special functions. This applies also to the four anterior cells, though they differ somewhat in their morphological character. The first cell looks as if it had a rounded shape. The next three cells have the aspect of pyriform elements with one process directed backwards. However, this shape shown in the fig. 1, *b*, Pl. 31, does not seem to render exactly the real form of the cells, as in some cases in better stained preparations more than one process arises from the cell (fig. 1, *a*, and 2, Pl. 31). The nucleus of the cells, rarely noticeable, is comparatively larger than the nucleus of the ganglion cells in the heart of Decapods.

Nusbaum, in 1899, described and figured nerve-fibrils in the peripheral part of the cell-protoplasm: "Im peripherischen Teile des Cytoplasmas findet sich ein zartes Fibrillennetz, welches den Kern umgiebt. . . ." As a matter of fact, the fibres which gave rise to this description can often be seen, but they do not belong to the cell itself since they are given off by the branches of the regulator nerves and only surround the cells with a kind of basketwork (fig. 6, Pl. 31; Text-fig. 9). The cells are embedded in a thick sheath of connective tissue which does not stain with methylene blue. Sometimes pale and indistinct fibres are noticeable which may be considered as belonging to this sheath; in such cases the surface of the cells may appear uneven and of a somewhat spongy consistency (fig. 3, Pl. 31).

The proximal parts of the two main processes have an uncommon structure, being made up of numerous fibres which originate separately from the cell but unite at some distance by twos or threes and finally make up one thick process. As shown in fig. 7, Pl. 31, the exit-points of these fibres occupy a good part of the surface of the cell. Before the junction into the common trunk these roots may give off short branches running to the sides (fig. 7, *a*, Pl. 31). Sometimes the different roots divide into two and unite again. Even the common trunk in its proximal part may be fenestrated in this way (fig. 7, *b*, Pl. 31).

All these features can certainly neither be regarded as caused by an accidental cleavage of the fibres or be considered as deductions from indistinct microscopical images, since these elements are well delimited, and can be seen in many preparations provided the methylene-blue staining of the nerves be not too deep. In the latter case the processes appear as though they were uniform up to the cells.

This interesting behaviour of the processes reminds one somewhat of the structure of the cells in the spinal ganglia of Vertebrates, the peripheric parts of which are fenestrated, and sometimes the process of the cells looks as if it began with several roots.

In the majority of the cells, from the fifth to the fourteenth namely, both processes have the same structure at their beginning. There is also no noticeable difference in their thickness except in the fourteenth cell, the posterior process of which is thinner than the opposite one, whereas in the fifth cell the anterior process is perhaps a little thinner. But this difference is not distinctly marked.

To each of the fourteen cells belong three kinds of projections: (*a*) short arborescences—the dendrites; (*b*) collaterals; and (*c*) long processes—the axons.

Dendrites.—We call dendrites as we did in our previous paper, short arborescences arising from the main two processes and sometimes from the cell-body. They can originate at various distances from the cell, some in its vicinity, others at a distance of 3 mm. or even more (fig. 11, Pl. 31). As to their number I counted up to twelve such ramifications belonging to the same neuron. They are doubtless homologues of similar outgrowths described in the Decapoda. In Squilla they are of various sizes and shapes (figs. 3–5, 7–11, Pl. 31). Their trunk may be longer or shorter, thinner or thicker. Sometimes the bush-like arborizations originate from the axon by means of two or three trunks (fig. 3, *d*, Pl. 31).

The characteristic ramifications of the dendrites end on the muscle-fibres (figs. 8 and 9, Pl. 31). The majority of the branches pass to the inner layer of the myocardium, but the superficial muscle-bundles (fig. 8, *ms*, Pl. 31) receive some fibres too.

As in Decapods, the dendrites may be accompanied by thin accessory fibres springing from the branches of the dorsal nerves (fig. 10, Pl. 31).

Collaterals.—We regard as collaterals the short and thin branches arising from the axons and the proximal parts of dendrites and ramifying in the ganglionic trunk (fig. 4, *col*, Pl. 31). It is difficult to ascertain, owing to their size and position in this trunk, how numerous they are and how distant from the cell may be the collaterals sent by one axon. They end in fine networks of fibrils which enter into relation with the fibres of the dorsal nerves and perhaps also with collaterals of other axons.

Axons.—As we have pointed out in the chapter concerning the general arrangement of the nerves in the heart-wall the axons of the cells just described form the ganglionic trunk. There is no doubt that these axons give off long branches running to the muscles, but in endeavouring to analyse the units of this local system, i.e. to trace exactly the fibres of every neuron, I failed in two important points.

Firstly, I could not make out how long the axons are, i.e. in which segment or segments a given cell has its terminal branches. The ganglionic trunk seems to be composed throughout its length, the anterior and posterior part excepted, of five to six axons in each transverse section. Nevertheless, the tracing of an axon at a longer distance has always been uncertain. To be sure, the long branches of the axon do not arise in the same segment in which the dendrites are given off, but at a greater distance from the cell. The branches seen in Text-fig. 3 do not therefore belong to the cell represented in the same figure but to a neuron the nucleated cell-body of which lies in one of the farther segments.[1] But in which of them? In some cases the axon seemed to branch in the third segment counting from the cell in which it originated, but there is no evidence that all other axons behave in the same way. It is worthy of note that the territory of the terminal branches of one neuron is evidently not confined to one segment only. This conclusion is drawn from the fact that side branches of two axons can be found in the same segment of the heart. The fibres of different provenance may run independently to the muscles or can build up a common nerve. Even four fibres branching at the same place from different axons have been observed.

Secondly, there is no decisive evidence as to the character of the second thick process arising from the cell. As was pointed out, both the main processes are at their beginning seemingly alike as to their mode of origin, their shape, calibre, and short arborescences. One of these processes is surely the axon, but what is the other? Is it to be considered as a second axon which takes its course in the opposite direction and branches in one of the segments lying on the other side of the cell as is hypothetically represented in the diagram (Text-fig. 5 A)? Or is it completely different from the axon, being only a thick trunk from which dendrites arise as seen in the second hypothetical diagram (Text-fig. 5 B)? Its shape, and the behaviour of its proximal parts, speak in favour of the first supposition; but the second one agrees better with the data obtained from the investigations of the same cells in Decapods. During my investigations on the nerve-cells in the heart of Astacus fluviatilis I have had as to the significance of their projections the same doubts which are expressed here. But after examining the same elements in the marine Decapods I could convince myself that they had one axon and several dendrites, one of which was often thicker than the others. In the Stomato-

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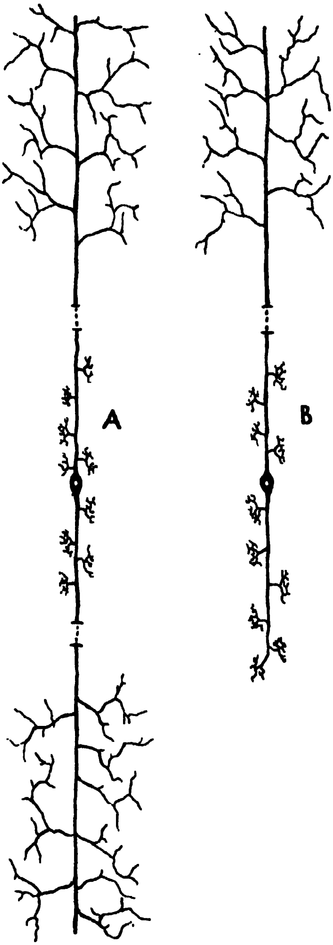
[1] Under 'segment' is meant here that part of the heart which corresponds to the distance between two lateral arteries.



TEXT-FIG. 5.
Explanation in text.

pods, too, the aspect of the four anterior and of the last posterior cells conveys the impression that these cells possess one axon only. I am, therefore, inclined to think that this is the real structure of the remaining cells also; but until the direct evidence be brought forward, i.e. until both processes can be traced up to their ramifications, this statement is not indisputable. Another difficulty arises in determining the direction in which the axon is sent from the cells. As to the four anterior elements their axons run most probably backwards, whereas those of the two posterior cells run to the front; but where are directed the processes of the remaining cells? We assume that one part of them runs forwards and the other backwards in some defined sequence. I have endeavoured to determine this direction by the angles at which arise the branches. These observations have but approximative value, since the branches may spring from the same axon at various angles. It looks, however, as if the anterior part of the heart was innervated by the axons coming from behind, and the posterior one by axons running backwards in the ganglionic trunk.

IV. NERVI CARDIACI DORSALES. SYSTEM OF REGULATOR NERVES OF THE HEART.[1]

1. General Arrangement.

There are three paired nerves which run from the central nervous system to the ganglionic trunk of the heart. Text-fig. 6 may facilitate orientation in the topography of the course of these nerves. On the right side of this figure the tergite is partly taken away in order to show the junction of the dorsal nerves with the ganglionic trunk, which takes place in the limits of the fourth segment of the body. The skeletal parts of this segment are described by Giesbrecht as follows: 'Das 4. Rumpfsegment hat ein weiches, durch eine quere Falte in 2 Wülste getheiltes Tergit, das von dem Hinterende des Rückenschildes bedeckt wird. An der ventralen Seite ist es stark gepanzert und zwischen den Maxillipeden des 2.–5. Paares mit medianen Kielen versehen.'

To this description I would add that this tergite contains parts of harder consistency which are useful for determining the topography of this region. In the posterior part there is a thin and elongated plate, *b*, which passes from the lateral to the dorsal side. This plate is often well marked by a distinct pigmentation. A second part of the tergite, *a*, which is of harder consistency, lies in front of the plate *b* at the proximal end of the Coxale of the second Thoracopod (raptorial limb).

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[1] The reasons for my using the terms 'dorsal' and 'regulator' nerves are given in the first part of my work. This Journal, vol. lxxv, 1932, p. 218.

All the three regulator nerves lie superficially on the dorsal muscles, and one ought to be careful when taking away the tergite as there is a danger of the nerves being torn away with it.

The following features concerning the situation of these nerves which we shall designate as nerves α, β, and γ are worthy of note[1] (Text-fig. 6).

The nerve α, taking an oblique course on the dorsal muscles, passes in front of the joint of the second Thoracopod.

The nerve β crosses the medial margin of the muscles near to the nerve α, but laterally the two nerves run at a greater distance from each other and the nerve β passes behind the joint of the second Thoracopod.

The nerve γ enters the heart at some distance from the nerve β, but laterally the two nerves lie near to each other though they do not unite into one bundle and the nerve γ runs on the posterior margin of the plate *b*.

It is not difficult to see these nerves as they are represented in the figure, for they stain easily and in a short time. It is sufficient to inject the dye under one of the abdominal tergites, and the dorsal nerves will appear deep blue sometimes already after thirty minutes. It is also possible to fix and mount preparations in which these nerves travelling on the dorsal muscles remain in connexion with the heart. The Text-fig. 7 is drawn on the basis of such preparations. As we see in this figure both nerves β and γ in their course on the thoracic muscles are incorporated in the branches of the thoracic nerves *Nn th* in which they travel from the central nervous system. I did not succeed in tracing exactly their proximal course, for the complicated chitinous skeleton in this region makes the dissections very difficult. It appears, however, evident that all the three regulator nerves spring from the large thoracic ganglionic mass. The branches which carry the nerves β and γ are most probably the nerves of the third and fourth Thoracomeres. As to the nerve *a* it belongs, of course, to one of the metameres situated anteriorly; but I am not able to state with any degree of certainty to which of them it ought to be assigned. Probably this gap in my observations could be filled up by the investigations of the larval forms or of specimens with softer chitinous parts.

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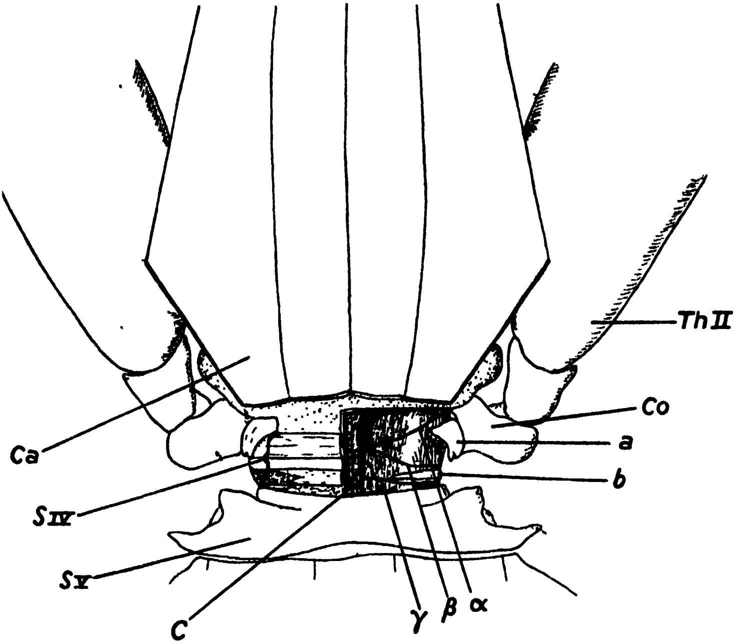
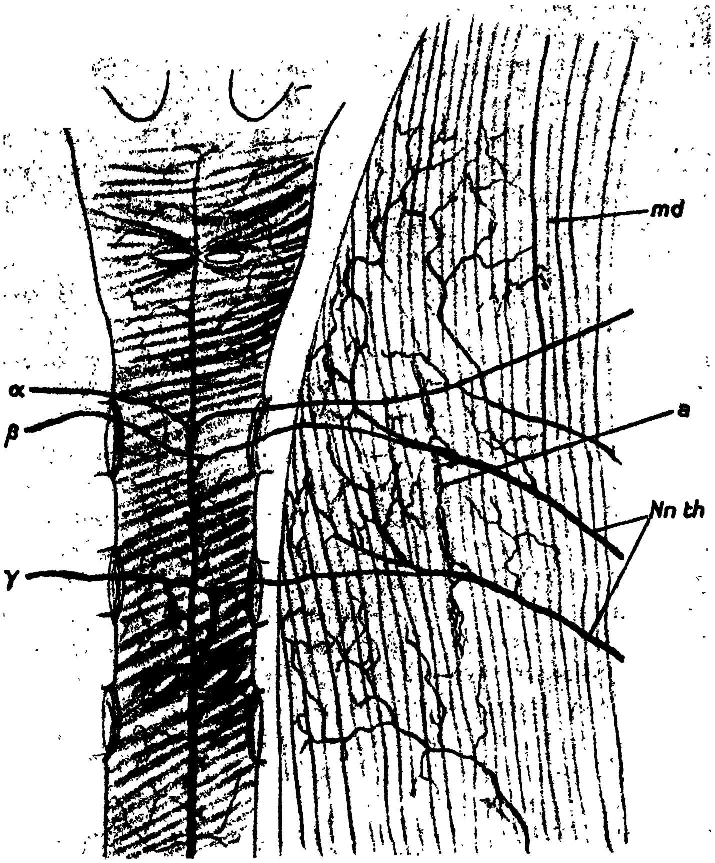
[1] I have chosen this designation by Greek letters instead of the more simple by the numbers 1 to 3 as in my previous publication I had used the term System I and II for designating two different sorts of fibres in the dorsal nerves. The homologues of these fibres are also present in the Stomatopods. As these two sets of fibres are conveyed to the heart by three nerves a confusion would easily result if the nerves were also indicated by numbers.



TEXT-FIG. 6.

Topography of the dorsal nerves of the heart. *Ca*, Carapace partly sectioned; *Th II*, second Thoracopod (raptorial limb); *Co*, Coxale; S IV, S V, fourth and fifth body-segment; *a*, *b*, plates which strengthen the chitinous parts of the fourth segment; *C*, heart; *α*, *β*, *γ*, dorsal heart-nerves.



TEXT-FIG. 7.

Junction of the dorsal nerves α, β, and γ with the ganglionic trunk of the heart. *Nn th*, branches of the thoracic nerves carrying the nerves β and γ; *md*, dorsal muscles; *a*, muscle-bundle with peculiar innervation in which nerve-cells are also taking part (not mentioned in the text). In the preparations from which this figure is drawn, and which are mounted on slides, the heart is brought to the same level as the dorsal thoracic muscles. The heart really lies deeper than the surface of the muscles, and in consequence the dorsal nerves turn downwards to reach the heart.

In the preparations stained with methylene blue the three nerves in question have not the same appearance. The nerve *a* is made up of one thick fibre only; arriving at the ganglionic trunk it divides into two branches of unequal calibre; one thinner runs forwards, the other thicker passes backwards.

The nerve β does not present the same features in all the preparations. Sometimes it looks as though consisting of one fibre only, in other preparations which are more completely stained this thicker fibre is accompanied by several thinner ones. The thicker fibre, on entering the ganglionic trunk, behaves in the same way as the nerve a, i.e. it gives off a small branch running anteriorly while the main branch passes backwards. The thinner fibres are of unequal calibre and partly break up in small granules. The nerve γ has, like the former, one thicker and several thinner fibres (Text-fig. 8). The latter stain in greater number than in the nerve β.

The composition of the nerves β and γ of several fibres could be observed only in that part of their course where they are not incorporated in other nerves. On their way with the branches of the thoracic nerves and in the ganglionic trunk these fibres can be traced individually but for a very short distance.

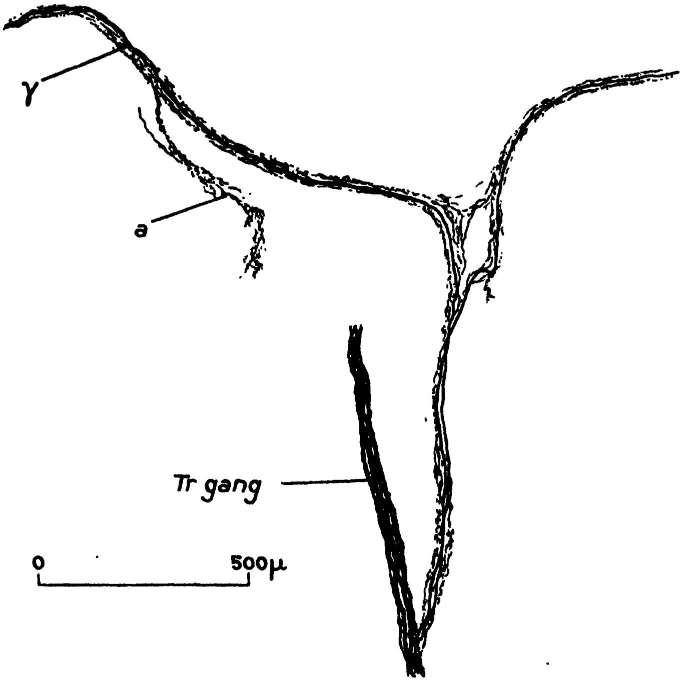
In some preparations the thin fibres of the nerve γ give off branches before entering the ganglionic trunk (Text-fig. 8, *a*). These branches correspond perhaps to the nerves of the pericardial cavity described in Decapods. The thin fibres breaking up in small beads show sometimes structures resembling those which stain so readily on the ligaments of the heart in Palinurus and Scyllarus.

All the three dorsal nerves are enveloped by a pretty thick sheath probably of connective tissue. However, in my preparations it does not stain with methylene blue and appears rather homogeneous.

2. Elements of the dorsal nerves in the ganglionic trunk and their relation to the elements of the local system.

There is no doubt that the elements of the dorsal nerves run throughout the entire length of the ganglionic trunk. The fibres of this origin are of various calibre, but even the stoutest do not reach by far the size of the axons of the local neurons;

their staining in a deeper shade of blue and their somewhat different outlines may also serve to recognize them among the elements of the ganglionic trunk, and help one to observe their course and connexions even in the posterior segments. On the other hand, however, as the tracing of each fibre separately throughout many segments is practically almost impossible, the origin of a given fibre from one or other of the dorsal nerves can only be suggested on the ground of some indications which we shall discuss later.



TEXT-FIG. 8.

Nerve γ joining the ganglionic trunk *Tr gang*; *a*, fibres branching from the nerve.

The aspect and the connexions of these fibres in the ganglionic trunk allow one to distinguish two sets of elements in them which are evidently homologues of those described by me in the

Decapods as System I and II, and which I shall now call by the same terms.

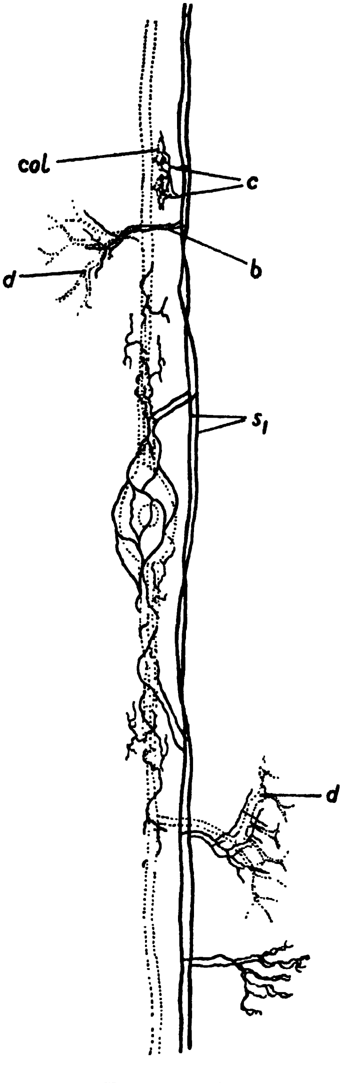
System I.

To System I can be referred the thicker fibres which enter into relation with the nerve-cells, their collaterals, and their dendrites. The diagrammatic Text-fig. 9 gives an idea of these connexions. The fibres, s_1, belonging to System I give off thin branches which have three fields of terminations. Some of them surround the cell of the local system, forming the basket-works already mentioned before, cf. fig. 6, Pl. 31. They stain readily, and are often deep blue when the cells are pale or even not stained at all. The fibres assume a beaded shape and sometimes expand to form leaf-like plates which are especially often stained on the most posterior cells. The fibres surrounding the cells pass also on to the main processes and interlace their 'roots'.

From the fibres s_1 arise, further, thin branches, *c*, ramifying in the trunk itself. They form fine networks of fibrils which are denser in the anterior part of the trunk, though large compact masses of the neuropile similar to those described in Decapoda could not be observed. This is evidently due to the elongation of the ganglionic trunk in Squilla. In the networks scattered in the trunk the terminations of different fibres of System I meet with one another as well as with the collaterals arising from the long processes of the cells.

The fibres of System I give off, finally, thin branches, *b*, going sideways from the trunk. They are nothing else than the 'accessory fibres' described above as accompanying the ramifications of the dendrites (fig. 10, Pl. 31).

In the anterior part of the heart, especially between the fifth and sixth cell, I have often observed branches of System I consisting of several thin fibres which run sideways and ramify on the muscles, apparently without connexion with the dendrites. As to their exact relations I am obliged to express the same doubts as when describing them in the Decapods, namely, I could not convince myself whether they are really independent of the ramifications of the dendrites or whether the latter are simply not stained at this place.

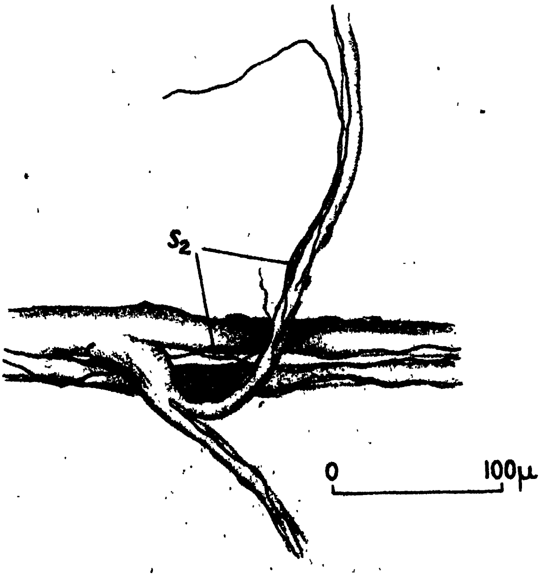


TEXT-FIG. 9.

Diagram showing the relation of fibres of System I, s_1, to a neuron of the local system; *col*, collaterals; *d d*, dendrites; *b*, fibres accompanying the dendrites; *c*, fibres ending in fine networks in the trunk.

System II.

System II consists of fibres thinner and more numerous than the main elements of System I, but they stain rarely and indistinctly, in consequence of which it is practically impossible to trace them at a greater distance. Travelling in the trunk the fibres in question branch successively sideways and run with the nerves of the local system. As shown in Text-fig. 10 one long branch of the axon may be accompanied by several thin fibres, s_2, of System II. Recalling what was said about the nerves arising from the ganglionic trunk, we come now to the conclusion that long branches contain (*a*) one or more fibres given off by the neurons of the local system, and (*b*) the fibres of System II of the dorsal nerves. The latter are of much smaller calibre, and look as if they were only supplementary elements in the branches of the trunk.



TEXT-FIG. 10.

Fibres of System II, s_2, accompanying the axons of the local system. Stained by the method of Gross.

Taking into account the above-mentioned difficulties of analysing the components of these branches, it might be objected that the thin fibres now considered as belonging to System II are simply the finer fibres of other neurons which send smaller branches to the nerves of the trunk. Of course, it is not easy to establish the criterion or distinguishing System II among other elements. Some help has been afforded by the methods of Cajal-Schultze and Bielschowsky-Gross since, after staining with silver salts, the fibres of System II become more deeply brown than the others, and their presence in the ganglionic trunk and in the thicker nerves can then be recognized with greater ease. Their small calibre may be considered as their characteristic morphological feature. Moreover, when tracing System II we do not notice the gradual decrease of the diameter of its fibres, whereas we notice it distinctly in the sub-divisions of nerves of other origin.

The question of the endings of these fibres could not be satisfactorily solved. As the nerves of the local system which are accompanied by the fibres of System II run to the muscles it is evident that the endings of System II are probably lying on the muscles too. Yet it is doubtful whether they always run in close apposition to the fibres of the local system, or have their own independent terminal branches. It is also a matter of doubt whether fibres of System II exist which pass to the muscles directly from the trunk without joining the nerves of the local system. Although such conclusions might be drawn from some preparations, I would still reserve my opinion upon this point owing to the possibility of various errors.

If we summarize what was said above we can state that the suggestion offered in our previous paper about the passing of the fibres of System II to the muscles has been strengthened by the observations of the same elements in Squilla, though precise data concerning the distribution of the terminal branches of this system cannot be given.

Turning now to the question of the origin of the two systems of fibres I and II we can consider as certain that they are carried to the heart by the three dorsal nerves α, β, and γ. But in which of these nerves run the fibres of each system? Direct observations, i.e. the tracing of fibres, are very difficult in Squilla since the elements of the main trunk are, especially in its anterior part, closely apposed to each other and, moreover, the staining of the individual fibres is, as a rule, not continuous, being often subjected to different interrùptions. We have therefore to arrive at some conclusion in an indirect way.

It is to be remembered that the nerve α consists of one thick fibre only, whereas the nerves β and γ are each made up of one thick fibre and several thinner ones. The thick fibres of all three nerves, so far as they could be traced in the trunk, behave in the same way. They send off branches which seemingly enter into close relations with each other just as do the fibres of System I in the Decapoda. Taking into account that, in the Decapoda, System I is composed of three thicker fibres on each side, it seems admissible that the same number of elements build up System I in Stomatopods. In consequence this system in Squilla would be made up of six fibres conveyed to the heart by all three paired dorsal nerves. In the ganglionic trunk, however, especially in its middle and hinder parts, these fibres do not stain in the same preparations all at once, and we find mostly two of them in one preparation.

As to the elements of System II they are given off, according to all probability, by the thinner fibres of the nerves β and γ.

According to this interpretation the nerve α carries fibres only of System I, whereas the nerves β and γ contain elements of both Systems I and II.

I tried to get more convincing evidence by investigating the effects of stimulation of the regulator nerves. To the excitation of the nerves β and γ by means of induced currents the heart responds with an acceleration of its rhythm. By the excitation of the nerves α the slowing of the heart has been obtained, but only in some experiments, while in others the stimulation was not effective. It may be that in the last case the excitability of the inhibitory nerves had been abolished in a very short time.

These experiments allow two interpretations. One may admit that the nerve α carries the inhibitory impulses while nerves β and γ are accelerator nerves. But the explanation is also admissible that the nerve α is an inhibitory nerve whereas β

and γ contain both kinds of fibres, i.e. the inhibitory and the accelerator ones, but that the action of the latter is predominating when the nerves are excited by means of the electrical current.

At any rate the fact that the nerve *a* which is made up of a thick fibre slows the rhythm of the heart, lends support to the suggestion that System I in the heart of the Crustacea has the same function as the vagus in the Vertebrate animals.

V. Nerves of the Arterial Valves.

As was emphasized in the general description of the nerves in the heart, the system of the valves consists of (*a*) the segmental nerves of the heart, and (*b*) the anterior cardiac nerve.

1. Nervi segmentales cordis[1] (Text-figs. 1 B, 11, and 12 B)

The segmental nerves originate in the ganglionic chain of the central nervous system, and in their proximal course are incorporated in the thoracic and abdominal nerves. Travelling with those branches of these nerves which pass on the dorsal side of the flexor muscles they leave here the somatic nerves and, independent of other elements, proceed to the heart. As they stain a deep blue colour it is possible to recognize them even in the somatic nerves, but I was unable to follow them separately up to the ganglionic cord. It is probable that in the central nervous system the neurons to which these nerves belong are in some relation with each other, but in their peripheral course the nerves of one segment have no connexion with those of the next segments. Only the valve of the last pair (XV) of the arteries has a common innervation with the valve of the posterior aorta. The independence of the nerves of each pair of valves, a feature in which the Stomatopods differ from the Decapods, is not difficult to ascertain owing to the distance of the valves from each other and the distinct staining of the nerves in the majority of them. However, the nerves in the four anterior arteries stain rarely and incompletely, and therefore, though their arrangement seems to be the same as in the other valves, I cannot consider it as established by strong evidence.

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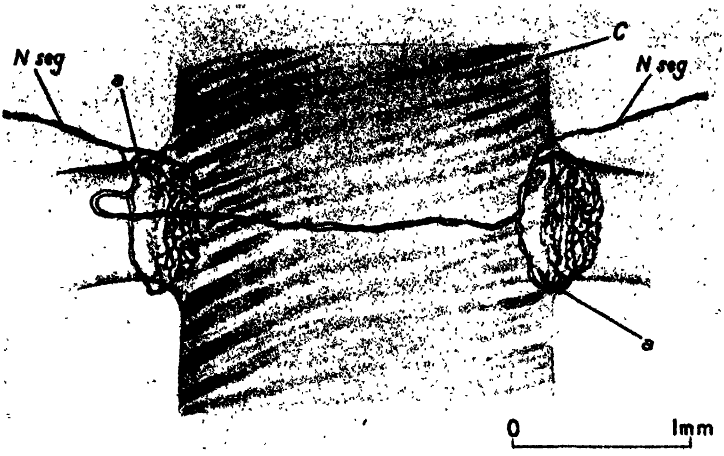
[1] This nomenclature might be found inappropriate since a part of these nerves do not correspond to the body-segments but to the metameric arrangement of the nervous elements. As was noted before the fourth body-segment is made up of the first four and a part of the fifth Thoracomeres. Nevertheless I have left this term 'segmental nerves', already used when describing homologous elements in Insects and in the Decapod Crustacea.

As the Text-fig. 11 shows, each segmental nerve, *N seg*, consists of a small quantity of fibres which give off characteristic branches dividing among the muscles of the valve. Before doing so each segmental nerve sends an anastomotic fibre to the contralateral nerve, and through it takes part in the nerve-supply of the opposite valve.



TEXT-FIG. 11.

Innervation of the valves of lateral arteries. *C*, heart-tube viewed from the ventral side; *N seg*, segmental nerves of the heart; *a*, leaf-like plates.

The arrangement of the nerves in the last pair of valves is represented in the Text-fig. 12 B. The nerves approach the heart running alongside the posterior aorta, *ao p*, where they give off anastomosing fibres and finally send off branches to the median and the two lateral valves. The course of the nerves running on the posterior aorta up to their origin could not be traced out. I think that they are nothing else than the last pair of segmental heart-nerves.

The branching and the endings of the fibres in the valves have the same appearance as in the Decapods. The thinner branches ramifying among the muscle-fibres of the valve present many varicosities or even are split into small granules. Larger leaf-like plates, *a* (Text-fig. 11), are also frequently present; they are mostly situated on the branches directed towards the surface of the valve. Whether or not they are artificial structures I cannot state decisively.

2. Nervus cardiacus anterior (Text-figs. 1 B and 12 A).

The anterior heart-nerve runs alongside the anterior median artery to end in its valve. The situation and the ending of this nerve are therefore the same as those of the nerve of Lemoine in the Decapods. In the latter animals the cardiac nerve has been described as belonging to the so-called stomatogastric system. In the Stomatopods the same system was investigated by Police in 1909, but the point of origin of the heart-nerve was not indicated by this writer. Further observations are necessary to elucidate this question as in my preparations the anterior course of this nerve is not sufficiently stained. Its endings are represented in the Text-fig. 12 A, in which we see that the anterior nerve, in the vicinity of the heart, divides into two branches which ramify when penetrating the anterior median valve. The terminal fibres on the muscles are of much the same appearance as in the remaining valves. No connexion has been observed between the branches of the anterior cardiac nerve and those of the segmental nerves.

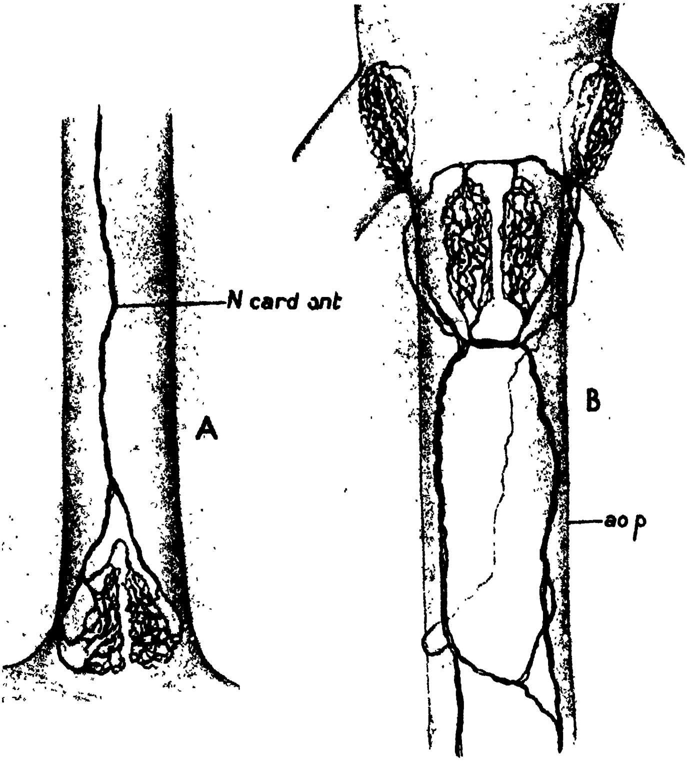
From the above description it results that so far as can be stated, the segmental nerves and the anterior heart-nerve do not give off branches to the wall of the vessels, but are destined for the innervation of the valves only.

Nusbaum (1899) claimed to have observed nerve-fibres and nerve-cells on the ventral subneural vessel of Squilla. I did not see these elements.

DISCUSSION.

I shall now endeavour to consider from the physiological point of view some points of the results described above.

With regard to the neurons of the local system, the assumption that the long branches are motor elements is the most probable of all. These branches, therefore, convey the impulses from the cells to the muscle-fibres. As to the short arborescent projections,



TEXT-FIG. 12.

A, Innervation of the valve of the anterior aorta. *N card ant*, Nervus cardiacus anterior. B, Innervation of the valves of the posterior aorta, *ao p*, and of the fifteenth pair of arteries.

I consider them as dendrites which carry impulses from the periphery of their ramifications to the cells or to the axons.[1] The fact that the endings of the dendrites are doubtless situated on the muscle-fibres has induced me to think that the contraction of the muscles acts as stimulus on the receptor parts of the neurons, and in the previous paper I have assumed that in this way a self-regulation of the neuro-muscular apparatus of the heart can be realized. Taking now into account the arrangement of the nervous elements in Squilla, it seems worth while to suggest that the excitation of the dendrites of a given neuron, caused by the contraction of the muscle-fibres, is propagated along the axon and its branches and on discharge causes a contraction of other muscle-fibres.

The course of the impulses according to our hypothesis is represented in the diagram Text-fig. 13 A. Suppose an impulse conveyed by the axon, *a*, starting from an element whose cell lies in one of the farther segments, causes contraction of the muscle-fibres m_1. This state of the muscles stimulates the dendrites of the cell *b*, and the resulting impulse is propagated through the axon to the muscles m_2 which contracting in their turn stimulate the dendrites of another cell, *c*. It is conceivable that by an appropriate distribution of the fibres the discharge of an impulse in any one of the neurons can involve the co-operation of the whole. In organs where the nerve-cells lie near to each other, their situation facilitates various interconnexions. In the long heart-tube of the Stomatopods, however, there must exist a special distribution of the nerve-elements, but I was able to decipher only a few features of this arrangement. As such, may be recalled here the behaviour of the axons, which in various segments most probably take the different course and, further, the fact that several axons send off their terminal branches to the same segment. Moreover, the situation of the dendrites of one cell is of such a kind that one may admit that the receptory area of each neuron embraces the muscle-fibres in a part of the

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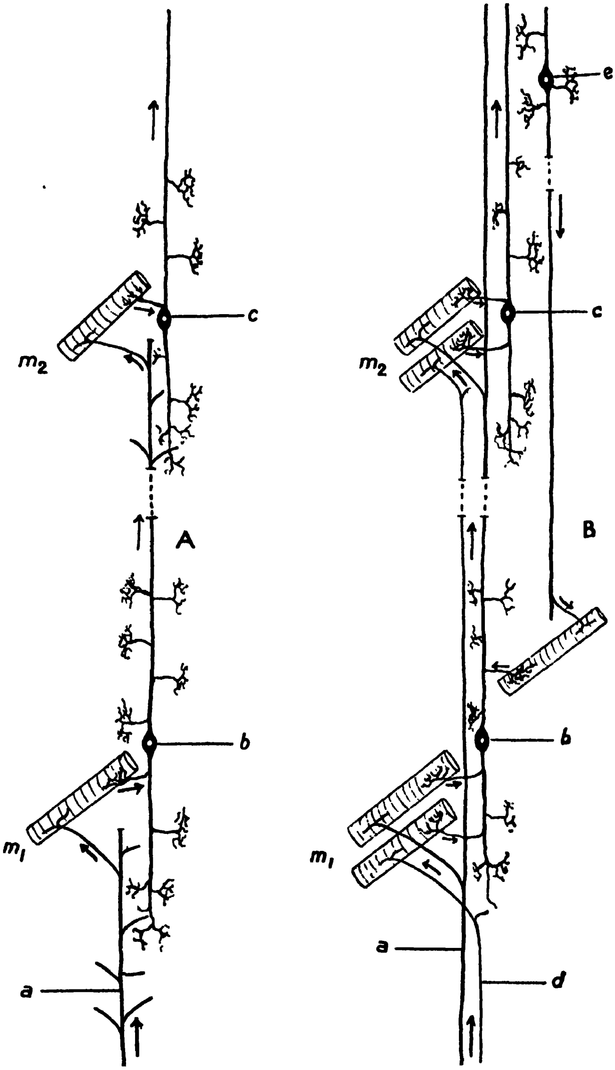
[1] We are compelled to add the words 'or to the axons' since a part of the dendrites arise from the axons and we cannot know whether the impulses conveyed by these dendrites pass through the nucleated cell-bodies or go directly to the branches of the axons.



TEXT-FIG. 13.

Diagrams showing the hypothetical course of impulses in the local system of the heart of Squilla mantis. The interruptions of the axons in the drawings show that these processes are long and do not end in the segments next to those in which lie the cells.

heart approximately equal to the length of one segment. The area of the motor-fibres of the same neuron does not lie in the same segment as the receptory area and seems to be larger, so that one neuron innervates the muscles of more than one segment. With respect to our hypothesis the observed facts would indicate: firstly, that one nerve-cell can receive impulses from different neurons since the muscles in which end their dendrites can be caused to contract in response to stimuli coming from more than one neuron; and, secondly, that one excited neuron can transmit the impulses to several others. According to this interpretation our second diagram, B, shows the neuron *b* receiving stimuli from the neurons *a*, *d*, and *e*, and the neuron *a* ending in the muscles m_1 and m_2, and in this way transmitting impulses to the cells of different segments.

If our suggestion would hold good for other organs endowed with autonomy of movement, we might conceive that in dependence on the arrangement of the nerve-elements, on the velocity of the propagation of the impulses, and on the quickness of the reactions which are taking place, the co-operation of the different parts of the organ can be either almost simultaneous or in other organs the contraction of the muscle-fibres can occur, as e.g. in peristalsis, in a definite succession.

The connexions of the neurons in the heart of Squilla appear still more complex if we take into account the networks given off by the collaterals. It is doubtful whether these neuropiles merely serve as fields of conjunction between the local system and System I of the dorsal nerves, or whether they bring also into relation the neurons of the local system with each other.

As regards the system of the efferent nerves which are acting as regulator fibres of the beat of the heart, we have already suggested that the thicker of them, termed System I, have an inhibitory function. They are in relation with the cell-bodies, their collaterals, and dendrites. Even admitting that some fibres of System I have another destination, the histological evidence seems to prove that the 'vagus system' of the heart in the Crustacea acts on the local neurons—on their cells and on their short projections.

This evidently does not apply to the second kind of regulator fibres, System II, which as we suppose conveys the accelerator fibres. They are provided with long branches of the local system ending on the muscles. As regards their action two ways can be imagined: these fibres convey the impulses either to the motor endings of the local system, or directly to the muscles. We should have to arrive at the latter conclusion were it proved that the accelerator fibres end on the muscles independently of the other nerves. There are some reasons for believing it, but strong evidence as to the exact course and connexions of these fibres cannot be brought forward.

The nerves of the valves are remarkable for their independence in the different segments in Squilla, whereas in the Decapods merely the nerve of the anterior aorta is unconnected with those of the remaining valves. The arrangement of these nerves in Stomatopods reinforces the supposition expressed by me in my previous publication, that the function of these nerve-elements consists in the maintaining of the tonic contraction of the muscles of the valves. One must conclude that in systole some factor must enter into action provoking the relaxation of the muscles and permitting the outflow of the blood into the arteries.

SUMMARY.

1. The three systems of nerves, viz. the local system, the regulator nerves, and the nerves of the arterial valves, which were previously described by the writer as innervating the heart of the Decapod Crustacea, have also been found in Squilla mantis.

2. The local system consists of not less than fourteen neurons. Their cells are situated in a nerve-trunk running alongside the dorsal surface of the heart, and, with the exception of the three anterior elements, lie at regular intervals each behind a pair of the ostial orifices. The cells give off the following processes: (*a*) the axons which form the chief part of the fibres in the ganglionic trunk and which after sending off many branches end on the muscle-fibres of the myocardium; (*b*) the dendrites—short arborescent branches arising both from cell-bodies and axons, and ending in the neighbourhood of the trunk on the

muscle-fibres too; (*c*) short collaterals ending in fine networks of fibrils in the ganglionic trunk.

3. The system of regulator nerves connecting the local system with the central nervous system, in the Decapoda consisting of one pair of nerves, is represented in the Stomatopoda by three paired nerves which in our description have been termed Nervi cardiaci dorsales. For the designation of each of them the letters α, β, and γ have been used. Their course indicates that they originate in the large thoracic ganglionic mass. After passing on the dorsal side of the extensor muscles these nerves approach the heart from its dorsal side, and enter its ganglionic trunk in the region of the fourth body-segment. The nerve α is made up of one thick fibre only, the nerves β and γ contain one thick and several thinner fibres each.

In the ganglionic trunk two sets of fibres given off by the dorsal nerves can be distinguished: one of them, termed System I, is made up of thicker fibres whose branches give synapses with the cells, collaterals, and dendrites of the local neurons; the other, called System II, consists of thinner fibres accompanying the long branches of the axons which pass to the muscles.

4. The system of nerves supplying the arterial valves is made up of (*a*) the anterior cardiac nerve running to the valve of the anterior aorta; and (*b*) the segmental nerves of the heart passing in each metamere to the valves of the paired arteries. There are, in all, fifteen pairs of these nerves. The last pair supplies the valves of the fifteenth pair of arteries and the valve of the posterior aorta. Each segmental nerve sends off anastomotic branches to the contralateral nerve, but does not show any connexions with the nerves of the neighbouring segments. In this respect these nerves in Squilla differ from those in the Decapods since in the latter they are all interconnected by anastomosing fibres. On the other hand, in Squilla as well as in Decapods the anterior cardiac nerve has no connexion with the segmental nerves of the heart.

5. With regard to the function of the nerve-elements enumerated above, the local system is to be considered as an autonomic apparatus which rules the beat of the heart, whereas the dorsal nerves convey the inhibitory and accelerator impulses from the

central nervous system. The first of the dorsal nerves, α, has been found carrying the inhibitory impulses. The stimulation of the two following nerves, β and γ, quickens the beat of the heart, but this effect of the physiological experiment does not exclude the possibility that the nerves β and γ contain both inhibitory and accelerator fibres.

The two sets of fibres in the ganglionic trunk which have been termed Systems I and II are probably concerned the former with the inhibitory and the latter with the accelerator action.

The function of the nerves of the arterial valves probably consists in the maintaining of a tonic contraction of the muscles of the valves.

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EXPLANATION OF PLATE 31.

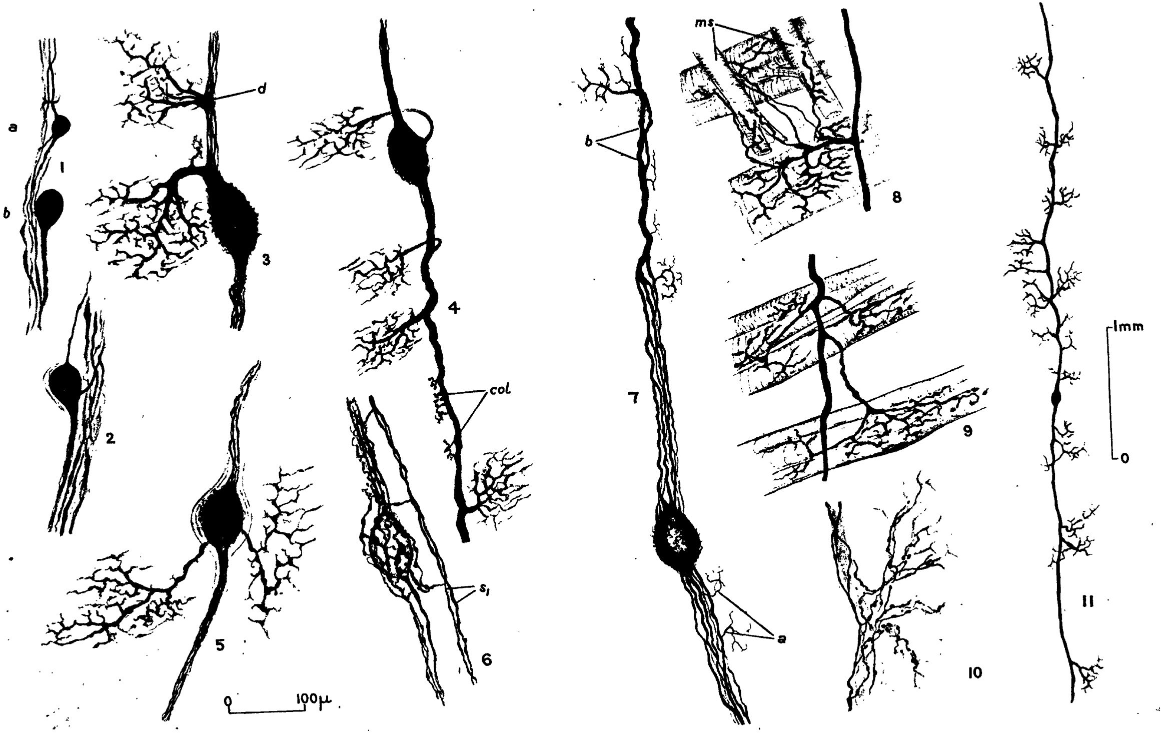
Figs. 1–7.—Ganglion cells of the heart of Squilla mantis. 1 *a*, *b*, 2, 3, the first, second, fifth, and eighth cell drawn from the same preparation; 3 *d*, dendrite arising by means of three trunks; 4, cell with three processes; *col*, collaterals; 5, cell with four processes; 6, the fourteenth cell with the pericellular network arising from fibres s_1 of the dorsal nerves; 7, ganglion cell with the proximal parts of its main processes made up of several fibres. The number of these roots is in reality greater than represented in the figure. *a*, branches arising from the roots; *b*, cleavage of the process.

Fig. 8.—Endings of a dendrite in two layers of muscle-fibres. *ms*, superficial muscle-bundles.

Fig. 9.—Endings of a dendrite in the muscles.

Fig. 10.—Accessory fibres which accompany the ramifications of a dendrite.

Fig. 11.—Nerve-cell with its dendrites.



J. S. Alexandrowicz, del.

Studies on the Cytology of the Harderian Gland of the Rat.

By

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With 9 Text-figures.

In a recent study by the author (1933 *a*, *b*) of the secretory process in various gland-cells, it was shown that (*a*) the secretory granules originated at the base of the cell in relation to the mitochondria, and later moved into the Golgi zone where maturation occurred, and (*b*) that development of the granules occurred in every case in intra-cellular vacuoles, these vacuoles being also the spaces inside which the vital dyes, Janus green and neutral red, are segregated. The present short study was undertaken, since it was noted that secretion formation in the Harderian gland-cell is different from that of gland-cells included in the former studies, and that in consequence certain points raised in the former work were in this case more clearly demonstrated. The purpose of the paper is then a description of the cytological elements of the gland-cell secretory granules, mitochondria, and Golgi apparatus, and a comparison of these structures with those obtained by vital staining with neutral red and Janus green.

The Harderian gland of the rat was used, the animals being killed by coal gas. Vital staining with both neutral red and Janus green was carried out supra-vitally at 37° C., using a 1/30,000 solution in Ringer of the former dye, and a 1/15,000 of the latter. Fixations used were 10 per cent. formol, Zenker formol, Da Fano, Mann's, and Champy's fixatives. Mitochondria were demonstrated by intensive staining using iron-alum haematoxylin, following the mitochondrial technique given by Cowdry (1918), i.e. 24 hours in 5 per cent. iron alum at 35° C., and 24 hours in 1 per cent. haematoxylin and glycerol after Champy fixation. The Golgi apparatus was demonstrated by Da Fano's method, and by Nassanow's (1924) modification of the Kolatchev technique.

HISTOLOGICAL APPEARANCE.

As described by various authors, Sundwall (1916) and Buschke (1933), the gland consists of two portions, an upper smaller lobule occupying the upper portion of the eye-socket, and a lower larger and more diffuse lobule occupying the floor of the optic cavity. As there is no marked histological difference between the lobules, they are here considered together. The histological appearance is that of alveoli with a wide lumen surrounded by cells completely filled with secretory granules. These granules are of a lipoidal character, and are not present in paraffin sections following non-osmic fixation. A membrana propria lies at the base of the secretory cells, and between it and the cells is a well-marked arrangement of basket cells. The lumina of the alveoli lead directly into the ducts.

VITAL STAINING.

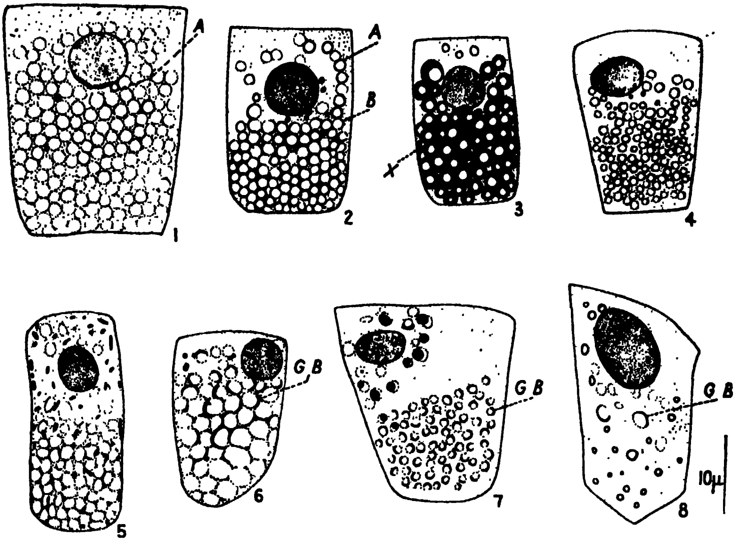
In the unstained gland the alveolar cells are seen to be filled with large numbers of highly refractile secretory granules. These granules are often extremely large, being 2·0–2·2μ in size. Many of the cells, however, contain smaller granules, and all stages are to be seen. When stained supra-vitally with neutral red, it is at once noticed that certain cells, about 10–15 per cent. of the total number, are selectively stained, so that under the low power those cells containing red-stained granules are easily visible through the gland. The appearance of the majority of the gland-cells is shown in Text-fig. 1. Even after heavy staining only one or two neutral red-stained granules A appear, and occasionally the periphery of one or two of the larger granules is also stained. Very often, however, these cells remain completely unstained. In the stained cells a remarkable condition is found. After very light staining, 2 minutes immersion in 1/30,000 neutral red solution, almost all of the formed granules are stained to some degree as in Text-fig. 2. This varies from a complete staining of the periphery as in granule A, to a partial peripheral staining as in granule B. In addition there are numerous smaller neutral red-stained granules which show a complete staining, though it is occasionally possible to focus a colourless granule inside. With more prolonged staining, 2–3 hours at 37° C., the appearance found in Text-fig. 3 is obtained. All the stained granules here show complete staining of the periphery, the stained area around the granule in all cases being much larger than that shown in Text-fig. 2. The irregularity of staining shown by some of the granules of Text-fig. 2 is still preserved, the red area on one side of the granule being often three or four times as wide as that on the other. Fusion between the red-staining portions as at X is also common. In addition, a few unstained granules still remain, these being probably fat granules, and are not related in any way to the secretory elements.

Staining with Janus green is found to give rise to two appearances. In some of the cells staining of the mitochondria occurs. These are small granules or short rods lying all through the cell, and are similar to those pictured by Sundwall (1916), and Beattie and McDonald (1933). They are shown in Text-fig. 5, which is drawn from a fixed preparation following the mitochondrial technique given. In Text-fig. 4, however, the more usual appearance is shown. As in the case of neutral red, certain of the cells show a well-marked staining of a portion of the periphery of the secretory granule, and in addition a number of smaller green-stained bodies. With Janus green, however, the complete periphery of the granule is rarely if ever stained. Partial staining, such as is usual in the more lightly neutral red-stained cells, is commoner, the stained area being in the shape of a small green crescent or batonette attached to one pole of the granule. By staining first in Janus green and then in neutral red double staining is obtained, the innermost portion of the batonette being green and the outer red.

Golgi Apparatus.

In the Cajal, Da Fano, and Champy techniques, all of which were used, an exactly similar type of Golgi apparatus was found. This is shown in Text-fig. 6 taken from a Da Fano preparation, and Text-figs. 7 and 8, which are Champy preparations. The Golgi apparatus consists in almost all cases of peculiar small crescentic bodies applied to the surface of the secretory granules. In smaller granules such as are present in Text-figs. 6 and 7, it may completely surround the newly forming granules A. In all cases, even after a very short treatment with 2 per cent. osmic acid, both the secretory granules and attached Golgi bodies blackened completely, and both Text-figs. 7 and 8 are drawn from cells strongly bleached by hydrogen peroxide. Of these, Text-fig. 7 is a cell in an advanced stage of refilling, the main mass of granules next the lumen being in the process of formation. Those granules around the nucleus are in all probability granules which have not been thrown out in the last discharge of the cell. Text-fig. 8 is a still earlier stage of secretory formation shortly after discharge, the smaller granules A, completely surrounded by a ring of osmiophilic material, being in an early stage of granule formation. With increase in size the osmiophilic ring is broken, the Golgi body being reduced to a crescentic body on the periphery of the granule. In completely matured cells the osmiophilic substance is no longer visible.

It is interesting to note in this connexion the work of various authors on lipoid secretory gland-cells. Heidenhain (1890) described in the pelvic and other glands of Triton a double appearance found in the secretory glands after ordinary fixation. Each granule apparently consists of two parts, the granule itself, a spheroidal stainable body, and a dark shell-like hood, the optical cross-section of which was a crescent. In a later paper by Fleischer (1904), he describes in the lachrymal gland of the ox peculiar crescentic structures applied to the surface of the granules as a cap, the Halbmondkörperschen. Sundwall (1916), working on the lachrymal gland of the same animal, was unable to confirm these findings. Bowen (1924) suggested a direct homology between these bodies and the osmiophilic bodies described by Nassanow (1923), and Bowen (1924), in various glands of salamanders. Ludford (1925) described similar crescentic bodies around the secretion granules in the sweat glands of the mouse following osmication, and identified these structures as Golgi bodies. In a later paper by Bowen (1926),



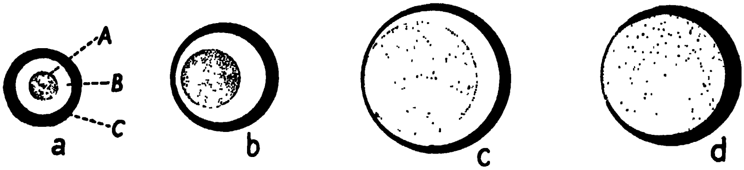
TEXT-FIGS. 1—8.

Resting cell of lachrymal gland of rat stained supra-vitally with neutral red. Two granules A show peripheral staining, the remainder being unstained. 2. Cell of lachrymal gland of rat during stage of refilling stained supra-vitally with neutral red. All of the contained immature granules show staining of their vacuoles. These vacuoles surround the granules partially or completely. 3. Cell of lachrymal gland of rat during stage of refilling after prolonged supra-vital staining with neutral red. Note the enlargement of these vacuoles and their tendency to coalesce as at X. 4. Cell of lachrymal gland of rat during stage of refilling stained supra-vitally with Janus green. Note staining of crescentic vacuolar spaces as in Text-fig. 2. 5. Cell of lachrymal gland of rat during stage of refilling showing mitochondria. Champy fixation. Iron-alum haematoxylin. 6. Cell of lachrymal gland of rat showing crescentic Golgi bodies, G.B. applied to granules. Da Fano fixation and silver impregnation. 7. Cell of lachrymal gland of rat in advanced stage of refilling. Golgi bodies are again crescentic. 8. Cell of lachrymal gland of rat in the early stages of secretion formation. Note circular Golgi bodies A around young granules, and transition to crescentic forms B in more mature granules.

an exactly similar appearance is shown in the cells of the oil-gland of the common fowl, and here again the crescentic structures are identified as Golgi bodies. In the Harderian glands of the cat, rabbit, and duck Bowen (1926) found the Golgi apparatus as a network lying at the periphery of the secretion mass, which is not extraordinary in view of the fact that the morphology of these glands varies from species to species. In both the cat and mouse which were investigated by the present author it was established beyond all doubt that these crescentic structures shown in Text-figs. 6, 7, and 8 were the Golgi bodies. In cells of the lachrymal gland of both animals osmicated for five to six days, nothing but the crescentic bodies were obtainable, and these were present mainly in the more immature cells. They were also the only structures found using silver impregnation following Cajal and Da Fano's fixatives. Identification of these crescentic bodies by any other than the osmic methods, as described by Fleischer (1904), was however difficult. At times in tissue fixed in formol it did appear that the crescentic bodies were revealed by basic stains though their small size rendered this difficult. It is thus quite probable that these osmiophilic bodies are homologous with the crescents described by Heidenhain (1890) and Fleischer (1904), though their small size in rodents renders identification by any other than the osmic or silver methods difficult.

DISCUSSION.

In drawing any conclusions from the above investigation the results following vital staining must be compared with those structures displayed in fixed preparations. In previous studies by the author (1933 *a*, *b*), it has been clearly demonstrated that neutral red and Janus green stain newly forming secretory granules, since both dye substances are segregated in the intracellular vacuoles surrounding these immature granules. Using this as a basis it is then clear that the cells showing selective staining with the dyes are cells in which secretory granules are being built up. In the Harderian gland of the rat this is apparently brought about by a simultaneous ripening of all the granules. Cells in which the secretion is fully formed, as in Text-fig. 1, show practically no staining of the granules since the perigranular vacuole has disappeared. With excess staining with neutral red the appearance found in Text-fig. 3 is obtainable, the granules showing a marked perivacuolar enlargement owing to excess deposition of the dye substance. The perigranular vacuoles of Text-fig. 8 are in every way comparable to the krinom bodies of Chlopin (1927), and to similar bodies induced by the author (1933 *b*) in the pancreas and salivary gland-cells. Their fusion, as at X in Text-fig. 8, would show that this may be a part origin of the large masses found in other gland-cells. The relation of these perigranular vacuoles to the Golgi bodies is of great interest. In the author's previous studies (1933 *a*, *b*), it was demonstrated in the various cells examined that the maturation of the granule, which was accompanied by loss of its surrounding vacuole, occurred inside the Golgi network. After excess vital staining which induces krinom deposits, the krinom which is the enlarged, fixed, and stained vacuole lay usually inside the network, the osmiophil strands of this surrounding it. This has been demonstrated by Ludford (1930), and has been confirmed by the present author. Krinom deposits lying outside the Golgi zone in the pancreas are not, however, in any relationship to the Golgi apparatus. It is clear then that in the case of perigranular vacuoles in the Golgi area the Golgi strands lie outside the vacuole, and in the case of the lachrymal gland it would seem that the probable development of a secretory granule in the Harderian gland is that represented in Text-fig. 9. As will be seen it is a modification of that originally given by Bowen (1929). In stage *a*, the granule A is first formed inside a vacuole B, in which vital dyes may be stored, as in Text-figs. 1–4. The vacuole is in turn surrounded by an osmiophilic ring C, such as are seen in the early stages in granule formation shown



TEXT-FIG. 9 *a*, *b*, *c*, *d*.

Schematic representation of stages in the development of a secretory granule in the lachrymal gland. A, Granule; B, Surrounding vacuole; C, Osmiophilic substance (Golgi body).

in Text-figs. 6 and 8. Enlargement of the vacuole as in Text-fig. 9 *b* and *c* is accompanied by a limitation of both osmiophilic substance and vacuole to one side of the growing granule, so that both in sections have usually a crescentic appearance. In this connexion it is of interest that Heidenhain (1890), in his pictures of the development of the secretory granules of the pelvic glands of Triton, distinctly pictures a space between the crescentic bodies and the secretory granule. Owing to the small size of these bodies in the rat this is difficult to see, except in vitally stained preparations, but there is little doubt that the space is the crescentic perigranular vacuole, and Heidenhain's drawing corresponds closely to Text-fig. 9 *c*. Later development, *d*, occurs through a disappearance of the vacuole as in more mature cells, the osmiophilic substance being limited to the crescentic body on one side of the now almost mature granule. It is not clear, however, what finally happens to this osmiophilic cap. Many mature cells show granules without it, and its later fate is uncertain.

CONCLUSIONS.

1. A study of secretion formation in the Harderian gland of the rat shows that the secretory granules are formed inside vacuoles, which may be selectively stained by Janus green and neutral red.
2. A comparison of the fresh and fixed preparations makes it probable that the Golgi bodies are in every case applied to the surface of the perigranular vacuole, throughout the development of the enclosed granule.
3. A scheme for granule development in this gland is advanced.

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On the Significance of the Enteronephric Nephridial System found in Indian Earthworms.

I. Evidence from their Habits and Castings.

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With 4 Text-figures.

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1. INTRODUCTORY.

IN 1919 (1) I described in the pages of this journal a new type of nephridial system found in Indian earthworms of the genus Pheretima. The nephridia in this system are remarkable in that they discharge their excretion into the intestine instead of on to the surface of the body. The excretion is conveyed through a system of ducts, of which the most important are a pair of longitudinal excretory ducts lying above the intestine, one on each side of the mid-dorsal line; these ducts open into the intestine all along its length through segmentally arranged apertures. I gave the name 'enteronephric' to this new type of nephridial system to distinguish it from the 'exonephric' type found in Lumbricus, and put forward the suggestion, originally made to me by Dr. W. N. F. Woodland, that 'the discharge of excretory fluid into the intestine may be a special adaptation for the conservation of moisture in a dry climate'. It was assumed that the water-content of the excretory fluid discharged into the gut was reabsorbed by the intestinal epithelium for the needs of the organism, while the solid excretory matters passed out of the body through the anus. Later, in 1924 (2), I found a similar 'enteronephric' system in another genus of Indian earthworms, Lampito (Megascolex), in which the nephridia are large and paired—meganephridia—like those of Lumbricus as contrasted with the numerous small micronephridia of Pheretima; but in the manner of their opening into the intestine through a pair of longitudinal excretory ducts and segmentally arranged apertures, these nephridia of Lampito agree with those of Pheretima. Still later in 1926 (3), I found that a third genus of earthworms called Woodwardiella also possessed an 'enteronephric' system very similar to what I had previously described in Pheretima and Lampito. I concluded, therefore, that this enteronephric type of nephridial system was widely prevalent amongst earthworms—particularly in the family Megascolecinae to which all the three genera possessing this type of nephridial system belong.

In fact, I went farther and suggested (2, 1924) that the basis of classification of Oligochaete nephridia should not be their size, as had hitherto been the case (micronephridia and meganephridia), but their place of opening—either to the exterior or into the gut. I am glad to find that Stephenson, in his recently published monograph on the Oligochaeta (7, 1930), has adopted my suggestion and has classified Oligochaete nephridia into the 'enteronephric' and 'exonephric' types, while retaining the terms meganephridia and micronephridia.

In order to determine the significance of this new type of nephridial system in earthworms, I have closely examined the suggestion that the enteronephric system is an adaptation for the conservation of moisture in a dry climate, and have tried to analyse the various factors in the life of these earthworms so far as they affect the problem of their peculiar excretory system, and have brought forward evidence to prove that the gut of Pheretima absorbs water efficiently, and that the peculiar type of excretory system is an adaptation for conservation of moisture.

My thanks are due to Mr. M. L. Bhatia for making photographs for text-figures 1–3.

2. HABITS OF PHERETIMA AS COMPARED WITH THOSE OF EUTYPHOEUS.

In order to appreciate the problem, it is necessary to know something of the conditions of life of an earthworm possessing the enteronephric type of excretory system. For this purpose the habits of the earthworm Pheretima were observed side by side with those of Eutyphoeus, which possesses an 'exonephric' system of nephridia. By comparing the habits of these two worms, with markedly different types of nephridial system, I arrived at tentative conclusions which were later confirmed by experiments.

The first fact to be borne in mind in this connexion is that the amount of moisture present in the soil is a very important factor in the life of an earthworm. In England and in Europe generally, the surface layers of the soil are generally moist during the greater part of the year, and the humidity of the air is also fairly high, so that earthworms are available almost at all times. But in Northern India, including the greater part of the Gangetic and Indus valleys, practically eight months of the year are dry and there is little moisture either in the air or in the soil during these months. The monsoon winds in Northern India blow for three to four months, i.e. during June (partly), July, August, and September, and it is only during these months that we get regular showers of rain. For the rest of the year, i.e. from October to June, there is practically no rain. After the monsoon is over, the water in the surface layers of the soil evaporates, the land gradually dries up, and there is very little humidity in the air. This dry weather continues for about eight to nine months except for an occasional shower or two in December or January. Of the eight dry months, May and June are extremely hot and dry; the hot winds (the loo) blow during these two months day in and day out; the land gets absolutely parched and baked, as it were, and the temperature goes up to between 110° F. and 116° F. in the shade. The moisture-bearing current breaks in generally towards the end of June or the beginning of July; the first few inches of rain are rapidly absorbed by the dry earth; but later, as the monsoon continues, the ground everywhere becomes wet; innumerable ponds and puddles are formed, and animal life shows its activity at its best. It will be interesting to add here that most forms of cold-blooded animal life—frogs and toads, lizards, snakes, tortoises and crocodiles, snails and slugs, centipedes and millipedes, and earthworms[1]—all breed in Northern India during the monsoon months, and not in the spring as they do in Europe. Here, therefore, it is moisture that largely determines the conditions of animal life.

Although earthworms are terrestrial in habit, moisture is absolutely essential for their cutaneous respiration, so that the skin of an earthworm must always be kept moist in order that it may work effectively in respiration, as otherwise the worms would die. Zoologists are agreed that these purely land-dwelling creatures have evolved from exclusively water-dwelling ancestors; but even though they have adopted a land habitat, it is apparent that the need for some kind of aquatic or semi-aquatic medium is still present.

The two earthworms—Pheretima and Eutyphoeus—not only possess two different types of nephridial system (enteronephric and exonephric), but also happen to be the most abundant earthworms in Northern India showing markedly different habits of life. Eutyphoeus is typically a 'rain-worm', as the German name 'regenwurm' for an earthworm indicates. It is a remarkable fact that the commonest earthworm found during the monsoon months in Northern India is Eutyphoeus, but as the monsoon ceases and the moisture in the soil diminishes, this earthworm disappears completely, presumably burrowing very deep into the ground and appearing only at the onset of the monsoon next year. During the dry months it is almost impossible to find specimens of Eutyphoeus. Compared with this worm, Pheretima, on the other hand, is very hardy and can be obtained almost throughout the year. During the monsoon, Pheretima, like Eutyphoeus, is of course ubiquitous, but during the seven or eight dry months when Eutyphoeus is nowhere to be found, Pheretima can be easily obtained wherever there is moisture in the ground. I have collected specimens of Pheretima in large numbers in gardens and near wayside water-taps even during the very hot and dry months of May and June. It would be a legitimate inference to draw from these facts that Pheretima does not need as much moisture as Eutyphoeus does; in other words, Pheretima is quite far removed from an aquatic life—at least much more so than Eutyphoeus. There is little doubt that Eutyphoeus needs soft wet ground such as can only be obtained during the monsoon months, while Pheretima can live in a comparatively drier and harder soil.

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[1] Most earthworms breed during or immediately after the rains. Eutyphoeus lays cocoons only during August, September, and October, but Pheretima lays cocoons not only during these two months but also in April, May, and June, wherever there is moisture in the soil.

Another important point of difference in the habits of the two worms is that while Eutyphoeus is a surface-feeder, Pheretima is a confirmed burrower. During the monsoon, Eutyphoeus is seen everywhere on the lawns and on the grounds feeding voraciously on the surface by picking up particles of earth with its anterior end; but one seldom finds Pheretima on the surface of the ground. Not that Eutyphoeus does not burrow, but it uses its burrow only as a place of protection. In fact, the worm spends the greater part of its life outside the burrow—it feeds and conjugates out in the open—and only keeps a foothold, so to speak, on its burrow. The tail end is retained in the burrow, so that at the slightest vibration of the ground, the worm immediately contracts and withdraws itself into its burrow. The burrow of Eutyphoeus is roughly U-shaped with two openings on the surface. Normally, the greater part of the body protrudes through one opening and lies flat on the surface of the ground as the worm picks up particles of earth and debris with its mouth; the other opening of the burrow is used for depositing its castings through the anus on the surface of the ground.

Pheretima, on the other hand, has a burrow with one opening only; it burrows with its anterior end and uses the opening of its burrow only for depositing its castings. It never feeds on the surface in the natural condition, but swallows the earth as it burrows. In the laboratory, however, it can easily be made to feed on moist blotting-paper. A feature specially suited to an underground life found in Pheretima and Megascolex (also in Plagiochaeta) is the presence of a continuous circle of setae in each segment. In an underground life there is an equal pressure all round the body, and therefore efficiency in progression is best attained by a 'continual leverage round the circular body'. Beddard (4, 1912) emphasizes that purely land-dwelling earthworms have evolved out of exclusively water-dwelling forms and further that the lumbricine arrangement of setae is a remnant of an aquatic life. The earthworm Eutyphoeus has a lumbricine arrangement of setae and this fact, coupled with its occurrence only during the monsoon months, leads us to the conclusion that this worm is suited to life in soft mud such as we find during the rainy months, or in other words, that it is still not very far removed from an aquatic life. Pheretima, on the other hand, is very far removed from an aquatic life and is more adapted to dry conditions, as it can and does dwell in comparatively dry stiff soil or vegetable debris and has a perichaetine arrangement of setae.

Another fact which tends to support the idea enunciated above is the occurrence of Pheretima to the exclusion of all other earthworms in almost pure sandy soil. Walking along a sandy river-bank during the monsoon months, one cannot fail to notice the castings of Pheretima to the complete exclusion of those of other earthworms. We know that sand can hold little water and that all the rain-water percolates through the sand to the deeper layers of the soil, in which the sand is mixed with a small quantity of humus and clay; still this almost pure sandy soil can accommodate Pheretima but no other earthworm. No doubt the worm burrows as far as it can to obtain nourishment and even moisture, but it must pass a good portion of its life in sand to deposit its castings on the surface. The remarkable fact emerges that Pheretima alone among earthworms can inhabit almost pure sand which holds little water and probably even little nourishment.

3. The Castings of Pheretima and Eutyphoeus.

A comparison of the castings of Pheretima and Eutyphoeus is both interesting and instructive. Charles Darwin

(5, 1881) has given beautiful diagrams of the tower-like castings which he believed to be the work 'probably of a species of Perichaeta'. Unfortunately, in his day, the genus Perichaeta was not very clearly defined, and systematists have now substituted the name Pheretima for the old name Perichaeta. Darwin relied for his information on Mr. John Scott of the Botanic Gardens of Calcutta, who wrote to him that 'these castings cause much trouble in the Botanic Garden, for some of the finest of our lawns can be kept in anything like order only by being almost daily rolled; if left undisturbed for a few days they become studded with large castings'. Darwin adds: 'These castings stand up like towers, with an open passage in the centre.' From the description given by Darwin and his diagrams, I have no doubt in my mind that the tower-like castings which he believed to be the work of a species of Perichaeta were really the work of Eutyphoeus and not that of Perichaeta (Pheretima). In Text-figs. 1 and 2, I have given Darwin's original figure side by side with a photograph of the castings of Eutyphoeus, and one cannot help recognizing the identity of the two castings. Eutyphoeus is a genus endemic in the Gangetic valley where, during the monsoon and after, 'the whole surface', as Darwin writes, 'becomes studded with castings' of this worm.

These tower-like castings formed by the vermicelli-like faeces of Eutyphoeus differ very markedly from the castings of Pheretima, which consist of very small rounded pellets or balls massed together at the opening of a burrow. Each rounded little pellet is distinct from the others and the worm voids these balls in succession during defaecation. A mass of such castings of Pheretima is shown in Text-fig. 3, and on comparison with Text-figs. 1 and 2, it will be seen that there is no similarity whatever between the castings of the two worms.

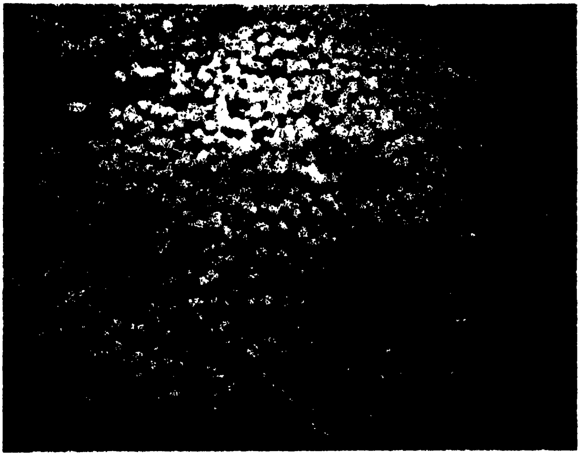
The mere shape and consistency of the two kinds of castings described above are significant. On examining the two types of castings at the time of defaecation, I have always noticed that while the faeces of Eutyphoeus are loosely semi-solid those of Pheretima are almost solid though moist. It is a matter of common experience that when our own faeces are semi-solid, and contain a quantity of water, the faeces tend to assume the form of a long winding string; but when the faeces are hard and solid and contain little water as in children, generally they tend to take the shape of rounded, oval or cylindrical pellets. These two kinds of faeces in our own case are found at different times, but the fact remains that the two types of faeces are really a result of the presence of different quantities of water in them. Similarly in Reptiles and Birds, not only are the faeces semi-solid but even the urine, which is first discharged into the cloacal chamber, is excreted out in a solid or semi-solid condi-

TEXT-FIG. 1.

TEXT-FIG. 2.

1. Darwin's figure (from a photograph) of 'a tower-like casting, probably ejected by a species of Perichaeta from the Botanic Garden, Calcutta'. (From Darwin's 'The Formation of Vegetable Mould through the Action of Worms, with Observations on their Habits', London, 1881.)
2. Photograph of a 'tower-like' casting of Eutyphoeus waltoni from Lucknow. This casting is closely similar to that shown in Text-fig. 1.

tion. The water is largely absorbed by the walls of the hind-gut and the cloacal chamber. The fact that Eutyphoeus always has long vermicelli-like castings while the castings of Pheretima always consist of solid rounded pellets is no doubt also dependent on the quantity of water present in their respective castings. On an analogy with the shape of human faeces, I concluded that in Pheretima the castings always contain less water than is present in the castings of Eutyphoeus. Just as in man and to a large extent in Reptiles and Birds, the large intestine and the rectum or the cloacal chamber absorb water, similarly the intestine and rectum of the earthworm also absorb water. It is probable that the intestine and rectum of Pheretima absorb water far more efficiently than do the intestine and rectum of Eutyphoeus, and that it is by virtue of this efficient absorptive capacity of the gut for water that Pheretima is able to live in drier conditions. That the other conditions are identical is clear from the fact that during the monsoon these two kinds of earthworms are found living side by side in the same soil and feeding on the same material but always



TEXT-FIG. 3.
Small, rounded, pellet-like castings of Pheretima posthuma (Perichaeta).

discharging two different kinds of castings in shape and consistency. Pheretima, no doubt, makes use of this absorbed water for its metabolic activities and is thus able to live in drier soils, while Eutyphoeus cannot live except in very wet ground. The only other explanation possible of this difference in the shape of castings can be the difference in size and efficiency of the sphincter muscle round the anal aperture. I have cut sections of the tail ends of the two earthworms and measured the thickness of the sphincters in the two cases, but I find that there is hardly any difference between the two. In fact, this was unnecessary, because on opening the rectal part of the intestine of Pheretima one finds that the faeces are already in the form of little balls or pellets, while the rectum of Eutyphoeus always contains a continuous column of faecal earth. When specimens of Pheretima are fed on blotting-paper for the purpose of cutting sections, even blotting-paper pulp is voided in the form of rounded pellets, and these pellets are found well-formed all along the rectum of the worm.

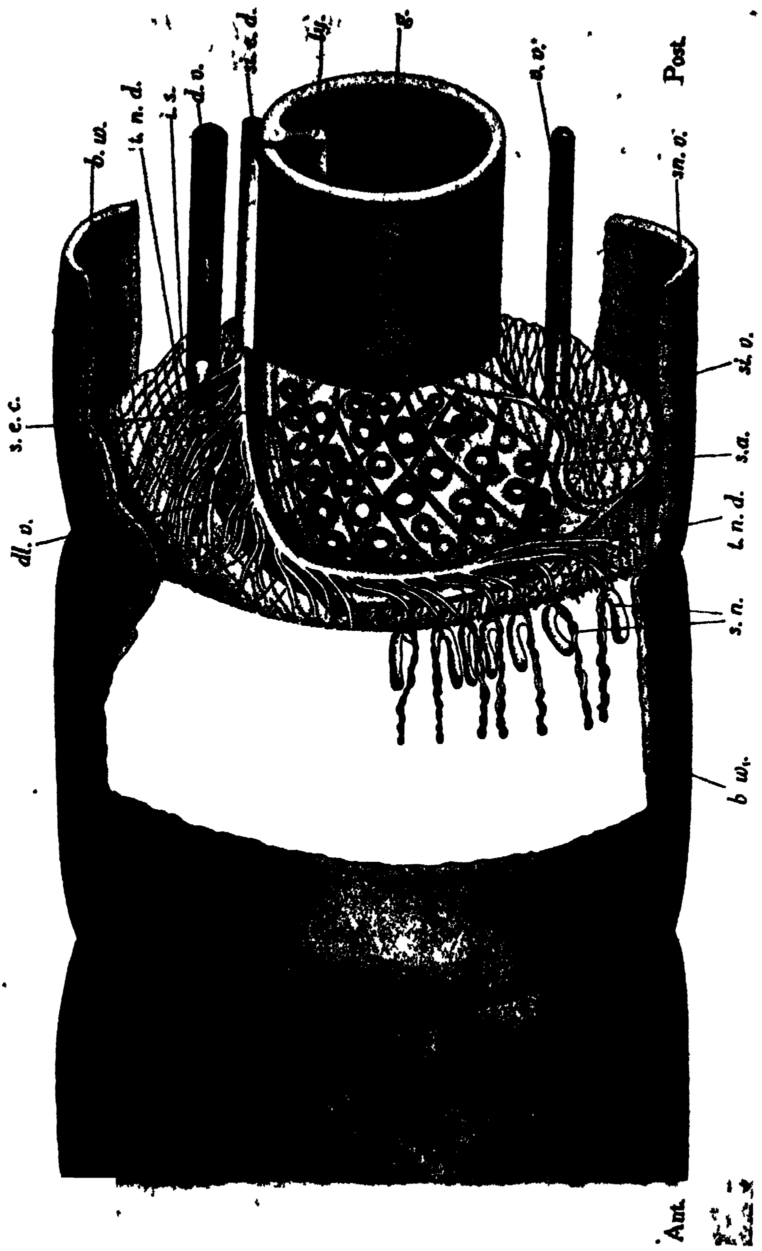
One more remarkable fact is worth mentioning here and that concerns the rate of feeding of these two types of earthworm. Under identical conditions, I collected their faeces and found that while in 15 hours the castings given out by a specimen of Eutyphoeus weighed 10·45 gms., those given out by a Pheretima weighed only 5·84 gms.—thus confirming my observation that Eutyphoeus is a voracious feeder as compared with Pheretima.

4. EVIDENCE FROM THE QUANTITY OF MOISTURE IN THE CASTINGS.

From an examination of the shape and consistency of the castings of Pheretima and Eutyphoeus, I came to the tentative conclusion that the intestine and rectum of Pheretima absorb water far more efficiently than do the intestine and rectum of Eutyphoeus. This conclusion is confirmed by an estimation of the percentages of water in the faeces of the two kinds of earthworm.

I have already stated that during the monsoon months these two genera live side by side in the same soil. The moisture contained in the soil is, therefore, the same for the two types. I naturally expected interesting results by comparing the percentages of moisture in their fresh castings. In order to avoid variations as to evaporation and differences in the soil, I cleaned about two feet square of the ground every evening in my garden, collected the castings of the two types early next morning before sunrise in separate closed jars, and immediately weighed the jars and the castings together. The empty jars with their stoppers had been previously weighed and it was, therefore, possible at once to know the respective weights of the fresh moist castings. The soil containing the two kinds of worms was the same and the evaporation was naturally lowest at night-time, particularly as the humidity of the air was at its maximum during the monsoon months. Unfortunately, castings for these weighings could not be collected on successive nights, since castings for purposes of comparison of moisture could only be collected when there was no shower of rain during the night. During 1930 I made five sets of collections and weighings. After determining the net weight of fresh castings, I dried them on a vapour-bath and weighed them; again dried and weighed them and so on till the weight became constant. This constant weight was noted and percentage of moisture was calculated in each case. The figures thus obtained are given in the table on page 571:

On comparing these percentages of moisture in the castings of the two worms, one finds that the difference ranges from 1·77 per cent. to 6·6 per cent. But in all the five experiments, the percentage of moisture is higher in the castings of Eutyphoeus than in the castings of Pheretima. This not only explains the shape and consistency of the castings of the two types of worm, but also confirms the conclusion that the gut of Pheretima absorbs water far more efficiently than that of Eutyphoeus, since the initial amount of moisture present in the soil swallowed by the worms was the same in both cases. This conclusion becomes still more striking and remarkable if we bear in mind the fact that the gut of Pheretima receives not only the moisture from the earth which it eats and from the digestive fluids secreted by the gut and its glands, as the gut of Eutyphoeus does, but that it also receives in addition



TEXT-FIG. 4.

	Weight of Fresh Castings in gm.	*Weight of Earth on drying on Vapour Bath in gm.*	*Moisture Evaporated.*	*Percentage of Moisture.*
			gm.	%
1st Experiment.				
1. Eutyphoeus	115·35	82·92	32·43	28·11
2. Pheretima	48·39	38·01	10·38	21·45
2nd Experiment.				
1. Eutyphoeus	81·244	63·811	17·433	21·45
2. Pheretima	71·757	57·161	14·596	20·34
3rd Experiment.				
1. Eutyphoeus	69·371	51·710	17·661	25·458
2. Pheretima	72·580	57·805	14·775	20·356
4th Experiment.				
1. Eutyphoeus	69·548	55·075	14·473	20·81
2. Pheretima	68·695	55·413	13·282	19·04
5th Experiment.				
1. Eutyphoeus	77·422	59·511	17·911	23·135
2. Pheretima	71·146	56·142	15·004	21·89

all the nephridial fluid from the septal and the pharyngeal nephridia which the gut of Eutyphoeus does not receive, as in the latter case, nephridia open to the exterior and not into the gut. Thus in spite of the fact that the gut of Pheretima receives extra fluid from its nephridia and therefore its castings should contain more water, actually the castings of Pheretima invariably contain less moisture than do those of Eutyphoeus. There can thus be no doubt whatever of the

Text-fig. 4 (*see opposite*).

A diagrammatic representation of the 'enteronephric' system and its relative position in Pheretima posthuma. Three segments are shown and part of the skin of the left side of the posterior segment has been reflected forwards to expose the various structures. *b. w.*, body-wall; *b.* w_1., body-wall cut and reflected forwards; *d. v.*, dorsal vessel; *dl. v.*, commissural vessel; *g.*, gut; *i. s.*, intersegmental septum; *s. a.*, sphinctered apertures; *s. e. c.*, septal excretory canal; *si. e. d.*, supra-intestinal excretory duct opening into the intestine at segmental intervals all along the length of the worm behind the fifteenth segment; *si. v.*, septo-intestinal vessel; *s. n.*, septal nephridia; *sn. v.*, subneural vessel; *t. n. d.*, terminal nephridial ducts; *ty.*, typhlosole; *v. v.*, ventral vessel. Each segment behind the fifteenth contains as many as 80 to 100 septal nephridia. From Pheretima by K. N. Bahl, 'Indian Zoological Memoirs', Lucknow, 1926.

extraordinary absorbing capacity for water possessed by the gut of Pheretima.

A reference to Text-fig. 4, showing the large number of septal nephridia of Pheretima, the septal excretory canals, the supra-intestinal excretory ducts and the segmental openings of the latter into the intestine all along its length, will convince any one that the excretory matter must be discharged into the intestine in a state of solution or else there is no meaning in the septal canals and the supra-intestinal ducts. Where does all this nephridial fluid disappear if, as we have already seen, there is less water in the faeces of Pheretima than in the faeces of Eutyphoeus? The conclusion becomes irresistible that the intestine and rectum of Pheretima absorb almost all the water present in the nephridial fluid discharged into the gut, and let the solid excretory matter pass out through the anus along with the faeces.

Recently, Wigglesworth (6, 1932) has put forward a similar hypothesis in the case of insects, in which he holds that the rectal glands and the rectal epithelium reabsorb water from the excrement before it is discharged to the exterior, and thus play an important part in the conservation of water.

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*Studies on the Germ-cell Cycle of Cryptocotyle lingua.

II. Germinal Development in the Larval Stages.

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With Plates 32–5 and 5 Text-figures.

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I. INTRODUCTION.

INVESTIGATIONS of the germ-cell cycle in the digenetic Trematoda, especially the more recent studies of Brooks (1930) and Woodhead (1931), have resulted in interpretations so opposite in nature that further study of this long-disputed question has become increasingly desirable. Previous explanations have, for the most part, been based on studies dealing with only the larval stages. For that reason, an interpretation of the entire life-cycle with reference to the concept of germinal continuity, as applied to trematodes, has been virtually impossible.

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* Parts I and II have been accepted by the Graduate School of New York University in partial fulfilment of the requirements for the degree of Doctor of Philosophy, June 1933.

A comprehensive study of the trematode germinal cycle involves some of the most difficult as well as fundamental problems of parasitology. Although many hypotheses have been advanced, it is not yet clear how the digenetic trematodes have acquired the present complicated life-histories. Was the vertebrate or the molluscan host the first to be parasitized? If the mollusc, did the early trematode become sexually mature in that host, thereby completing the life-cycle in a manner similar to the free-living and ectoparasitic flatworms, or did it immediately acquire one or more intercalary stages similar to those in the present molluscan host? If the first host was a vertebrate, how did the mollusc and other intermediate hosts become involved? To what extent has germinal development changed with the evolution of the parasite and modification of the life-cycle? Are the interrelationships of various trematode families sufficiently close to expect the group as a whole to be characterized by the same type of germ-cell cycle? Are there to be found among free-living or parasitic turbellarians, or in the life-histories of the Monogenea, any stages or modes of development such as polyembryony, internal budding or parthenogenesis, that foreshadow or correspond to the intercalary stages of the Digenea? If not, are we justified in assuming then that endoparasitism alone is responsible for these larval stages?

It is believed that satisfactory answers to these questions must come from careful and precise studies dealing not only with the entire cycle of a number of representative digenetic trematodes, but also with other forms which are evidently related to this group, particularly the rhabdocoele turbellarians, monogenetic trematodes, and the Mesozoa (Rhombozoa).

The morphology and parasitic habit of certain of the rhabdocoele turbellarians are suggestive of the Trematoda. In form and habit, Anoplodium, an endoparasite of holothurians, and Graffilla, from the mantle chamber of molluscs, have been regarded as similar to the ancestors of the Digenea and Monogenea respectively. The life-history of Anoplodium, however, is direct, and larval stages similar to those of the Digenea are lacking.

In development and life-history, similar phenomena have been described for both turbellarians and trematodes. Polyembryony, which has been reported for both monogenetic and digenetic trematodes, has also been described for certain of the turbellarians in which two or more embryos (two or three in Graffilla to as many as thirteen in Syndesmis) develop in a single egg capsule. Linton (1910), in his studies on Graffilla gemellipara from the mussel, Modiolus plicatulus, believed that the two or three embryos contained in each capsule came from a single fertilized ovum. This turbellarian was probably mistaken by Nicoll (1906) for a trematode sporocyst, as pointed out by Patterson (1912) who concluded, in regard to its development, that the twin embryos result from the enclosure of two fertilized ova in each capsule and not from the division of a single ovum or embryo.

There are features in the life-cycle and germinal development of Dicyema (Whitman, 1882; Hartmann, 1925), and perhaps other Mesozoa, which may throw considerable light on the interpretation of the digenetic cycle. The Mesozoa are chiefly parasites of cephalopods, although orthonectids infest turbellarians, nemertines, annelids, and echinoderms. The group as a whole has been regarded by certain zoologists as intermediate in position between the Protozoa and the Metazoa. The bulk of evidence indicates, however, that the Mesozoa are flatworms which have become degenerate as a result of parasitism. Certain stages of their life-cycles resemble trematode miracidia and sporocysts, particularly with regard to their germinal development, as pointed out by Reuss (1903). The early cleavage stages of the mature agametes of Dicyema, and of the mature germ-cells of Cryptocotyle rediae, are quite similar in nature. These and other cytological resemblances will be discussed later in connexion with germinal development of the trematodes.

Although the relation of the monogenetic trematodes to the Digenea is a matter of considerable controversy, and is beyond the scope of the present investigation, the life-histories and modes of reproduction in certain monogenetic species are of interest to this study. The developing young of Gyrodactylus elegans were observed by Wagener (1860) and other early investigators to contain embryos of a third generation while still within the body of the parent. Von Linstow (1892) believed that the secondary embryos resulted from asexual reproduction in the primary or daughter embryo. Metschnikoff (1870), however, insisted that the secondary embryo has the same origin as the primary, namely, the segmentation products of the fertilized egg; the primary and secondary embryos would accordingly be interpreted as 'sisters' rather than 'mother and daughter', since they are of the same age. Kathariner (1904) supported Metschnikoff's opinion and, in a later paper (1920), applied his findings on the monogenetic species, Gyrodactylus elegans, in a review of life-histories and germinal continuity in the digenetic trematodes.

As a rule, endoparasitic trematodes have digenetic life-cycles while ectoparasitic forms are monogenetic. Certain of the Aspidocotylea, however, are exceptions to this generalization in that they are true endoparasites and, as far as is known, are monogenetic in development. Species of Aspidogaster have been described from the coelomic cavities of molluscs and the intestine of fishes and turtles. It seems quite possible in this case that Aspidogaster is typically parasitic in bivalves, and is merely carried over into the vertebrate when the mollusc is eaten. It is further possible that the occurrence of Aspidogaster in vertebrates represents a case of incipient endoparasitism. Leuckart, however, regarded the aspidogastrids as sexually mature rediae whose former adult stages had been eliminated.

The relation between habitat and life-cycle is further complicated by the observations of Gallien (1932) on Polystomum integerrimum, whose developmental cycle, according to him, consists of two distinct generations. The first is ectoparasitic on the gills of young tadpoles and produces a second generation which becomes attached to the gills of older tadpoles. Upon metamorphosis of the host, the polystomes pass down the alimentary canal and become established as endoparasites in the urinary bladder of the frog. This is a case, therefore, in which an apparently digenetic life-cycle, consisting of an ectoparasitic and an endoparasitic generation, involves the larval and adult stages of a single host species.

While some of the earlier investigators regarded the Monogenea as close to if not directly in the phylogenetic line of the Digenea, Leuckart's opinion concerning the Aspidocotylea would derive that group at least from the digenetic trematodes. Since the Monogenea are largely ectoparasites of vertebrates and have direct development, it is possible that they adopted the parasitic habit at a comparatively recent time, after the appearance of vertebrates, and therefore that they are not closely related to the older digenetic group.

The present paper completes, as far as is possible with the material at hand, a study of the germ-cell cycle in Cryptocotyle lingua Creplin, a digenetic trematode belonging to the family Heterophiidae; gametogenesis in the adult has been described in an earlier report (Cable, 1931). Specific investigations to determine the origin and differentiation of germ-cells, the occurrence of maturation or other sexual phenomena, and germinal continuity in the larval stages have been undertaken. Stunkard (1930), in his studies on the life-history of Cryptocotyle, did not succeed in hatching the eggs, and stated the belief that unhatched eggs are eaten by the snail. Since the writer has not had an opportunity to test this hypothesis, one stage of the life-history, the miracidium-mother-sporocyst, has not been observed. It is surprising that at least recognizable fragments of this stage were not found in the dozens of infected snails that were carefully examined in serial sections during the course of the study.

The life-history of Cryptocotyle may be described briefly as follows. The adults inhabit the small intestine of fish-eating birds and mammals. Feeding experiments have demonstrated a low grade of specificity for these hosts. Eggs pass out with the faeces, and if they reach sea-water, miracidia develop within about ten days. The marine snails, Littorina littorea and Littorina rudis, serve as molluscan hosts, in which there is a single redial generation. Cercariae escape from the snail and encyst in the skin of the cunner, Tautogolabrus adspersus, and other fishes. When the infected fish is eaten by a suitable bird or mammal, the cyst-wall is ruptured and the young worm develops to sexual maturity in about five days.

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II. Historical Survey.

Many theories and interpretations of the germinal cycle of digenetic trematodes have been advanced, but none of them has become generally accepted. Brooks (1930) classified these hypotheses as follows: metagenesis, heterogeny, paedogenesis, extended metamorphosis and germinal lineage with polyembryony. To this list may be added Woodhead's (1931) concept of polymorphism with three sexually mature hermaphroditic generations.

The theory of metagenesis, or alternation of a sexual with an asexual stage, was applied to the trematodes by Steenstrup (1842), who likened their development to that of the coelenterates and tunicates. This view was supported by Moulinie (1856), Pagenstecher (1857), Wagener (1866), Balfour (1880), and Biehringer (1884). Since the first trematode life-history, that of Fasciola hepatica, was not traced experimentally until 1883, the opinions of these earlier investigators were based on homologies of larval and adult stages.

Grobben (1882) first suggested that heterogeny (alternation of a bisexual with a parthenogenetic generation) occurred in the Digenea, and expressed the belief that cercariae develop from parthenogenetic ova. The germ-cells of sporocysts and rediae have been the object of several more recent investigations, the point in question being chiefly to determine whether or not polar body formation or other maturation phenomena are associated with these cells. Reuss (1903) observed, in the sporocysts of Distomum duplicatum, that the 'ovum' was accompanied by three smaller cells which he interpreted as polar bodies. Haswell (1903), in his studies on two sporocysts occurring in Mytilus latus, stated in a footnote that he did not confirm the findings of Reuss, but observed that 'in a large proportion of the specimens there occurs lying loose in the sporocyst in the immediate neighbourhood of the ovary a varying number of cells (fig. 14) which have homogeneously deeply staining nuclei 0·002 mm. in diameter. If these are not of the nature of polar bodies it seems difficult to account for them'. Haswell also reported that the ovary, after considerable division of its component cells, became fixed in position and completely enclosed in a delicate membrane. The ova gave rise to both cercariae and daughter sporocysts. Upon leaving the mother sporocyst, the daughter sporocysts, often containing cercarial embryos, were observed to divide by binary fission.

Tennent (1906) reported finding polar bodies in the sporocysts of Bucephalus haimeanus, from the gonads of the oyster. He described two methods of germ-cell origin, both of which were from the wall of the sporocyst. In the first, there seemed to be no definite place of origin; in the second, germ-cells were produced by a definite 'keimlage'. In the older germ-tubes of the branching sporocysts, cell-boundaries disappeared and the cytoplasm condensed around the nuclei, forming germ-cells which passed into the lumen of the tube. In a few cases, Tennent described the cutting off of a small cell, after which the nucleus moved to the periphery of the larger cell and divided unequally to form a second small cell. Cleavage followed this process, and the germ-balls were of considerable size when they left the keimlage and entered the lumen of the sporocyst. Tennent also reported finding central opaque masses in the young sporocysts.

Cary (1909) studied germinal development in a sporocyst which he thought was that of Diplodiscus temporatus. It has since been shown by Cort (1915) that Cary confused two distinct species, but it is of interest to note that in the form with which he worked Cary reported that germ-cells developed from cells of the body-wall. In the process of maturation, a single polar body was extruded without a reduction of chromosome number. Faust (1917) reported the occurrence of true parthenogenesis in the rediae of Cercaria flabelliformis. He observed that the 'ovum' extruded a single polar body which sometimes divided. Chromosome reduction did not occur. He refuted the arguments of Rossbach against polar body formation, stating, "The polar bodies have been found not only in cytoplasmic continuity with the ovum, but in the actual stage of mitosis preceding the separation of the polar nucleus from the germ-ball". He described a specialized germinal mass at the blind end of the gut as the source of germ-cells. In simpler types such as Cercaria diaphana and Cercaria micropharynx, he reported that the entire layer beneath the epithelium was germinal in nature.

A large number of workers, on the other hand, have been unable to find evidence of maturation in larval trematodes, and hence maintain that heterogeny is not characteristic of this group. Looss (1892) did not report the occurrence of polar bodies in the larval stages of Amphistomum subclavatum. In his figures are shown small, densely-staining nuclei in the germ-balls which he designated as "degenerierende Kerne (?) der Keimballen". The degeneration of entire germ-balls is also figured by Looss. He described the production of germ-cells both from a definite keimlage and from cells of the body-wall. In the latter method the cells of the wall divided, one of the daughter cells remaining in place, the other being cut off into the lumen where it developed into a germ-ball.

Coe (1896) found no polar bodies in the sporocysts and rediae of Fasciola hepatica. His material, however, was unfavourable for study and he preferred to draw no conclusions. Rossbach (1906), working on Cercaria armata and Cercaria echinata, observed cells similar to those interpreted as polar bodies by Reuss (1903). Rossbach, however, concluded that these small cells belonged to the soma of the sporocyst or redia, and had nothing to do with the germinal development. They were not in direct continuity with the germ-cells, present often in larger numbers than threes, and were found in all stages of development, including the ovary of sexually mature adults. Mathias (1925), in his studies on the developmental cycles of Strigea tarda, Hypoderaeum conoideum, and Psilotrema spiculigerum, did not observe polar bodies or other maturation phenomena. Dollfus, in a private communication to Brooks (1930), stated that he had never found indisputable polar bodies in the thousands of sporocysts and rediae which he had examined. Among the material investigated by Dollfus were the larval stages of Bucephalus haimeanus, the same species for which Tennent (1906) described maturation phenomena. Dubois (1929) disagreed with Dollfus, claiming to have observed germinal discontinuity and parthenogenesis in the sporocysts of Cercaria helvetica V.

The concept of a metamorphosis extending over several generations was supported by Balfour (1880), Leuckart (1886), and Looss (1892). Balfour outlined the theoretical life-history of a typical trematode, stating that the majority of its stages "are simply parts of a complicated metamorphosis, but in the coexistence of larval budding (giving rise to Cercariae or fresh Rediae) with true asexual reproduction there is in addition a true alternation of generations". Looss (1892), in expressing a similar opinion, homologized the sporocyst, redia and cercaria, pointing out that they possess the same general structure. The concept of extended metamorphosis was strengthened by the early studies on the monostomes in which the miracidium contains a single fully formed redia at the time of hatching, and, for that reason, was likened to Desor's larva of the nemertines. This condition has also been described by Linton (1914) for Parorchis avitus, a distome species from gulls.

Germinal lineage, both with and without polyembryony, has been applied to the trematode life-cycle by a number of workers. Leuckart (1886) believed that the germ-cells of sporocysts and rediae are derived directly from the egg, and remain in an undifferentiated condition in the body-cavity of sporocysts and rediae during their somatic development. Various modifications of this theory have been accepted by Thomas (1883), Schwarze (1885), Coe (1896), Dollfus (1919), Kathariner (1920), and Brooks (1928, 1930). From his studies on the larval stages of several distome and monostome species, Dollfus (1919) concluded that sporocysts, rediae, and cercariae do not develop from the wall of sporocysts and rediae, but from one and the same germinal line which comes from the segmentation of the fertilized ovum. The cells of this line give rise to the larval somatic tissues of the rediae and sporocysts by a sort of internal polyembryony. These somatic tissues are merely larval envelopes which have enclosed the cells of the germinal line and, being sterile, take no part in reproduction. Kathariner (1920) also supported the theory of germinal lineage, but maintained that polyembryony in sporocysts and rediae was only apparent since the germ-cells were not derived from but merely enclosed by the soma of these stages. The germinal line would accordingly be uninterrupted from fertilized egg to fertilized egg.

From his studies on the larval development of twenty species of trematodes, Brooks (1930) interpreted the life-cycle 'as being one where the germinal lineage passes through successive larval stages in which polyembryony features as a mode of multiplication and in which precocious cleavage of the germ-cells is the activating factor'. According to Brooks, the primordial or 'antecedent' germ-cells which are found in the body-cavity of the miracidium-mother-sporocyst divide to form loosely organized 'germ-masses'. These dissociate and their components may by division form 'secondary germ-masses' or may develop into germ-balls. 'Tertiary germ-masses' may possibly be produced by the components of the 'secondary germ-masses'. The dissociation of these masses was interpreted by Brooks as typical polyembryony. He maintained that sporocysts and rediae are homologous, the extent to which their somatic structure is developed depending upon the intensity of the factor for precocious cleavage. In the germ-masses, this factor is so strong that no observable somatic structure is formed, while in the development of daughter-sporocysts, rediae, and daughter-rediae, the tendency toward precocious cleavage being progressively decreased, somatic expression becomes correspondingly greater. The precocious tendency of the germ-cells eventually becomes decreased to such an extent that the embryos are able to undergo 'natural' development, producing larvae (cercariae) in which the germinal elements are normally restrained. Since polyembryony has been experimentally produced in a number of forms by lowered oxygen tension and changes in other factors such as temperature (Stockard, 1921) and food (Marchal, 1904), Brooks suggests that the cause of polyembryony in trematodes may likewise be exogenous in nature. Since a reduced oxygen supply may produce polyembryony, he reasons that this phenomenon may have occurred in the digenetic trematodes on adoption of the parasitic habit in which less oxygen was available than in the free-living state. Brooks did not find polar bodies or structures that could be interpreted as ovaries.

Polymorphism has recently been advanced by Woodhead (1931) as an explanation of the life-cycle in the gasterostomes. He interpreted the sporocyst and redia, which he has described for the first time in this group, as adults rather than larvae, and has described gametogenesis and fertilization in these stages as well as in the final adult gasterostome. Woodhead concluded, accordingly, that Bucephalus is polymorphic, the life-cycle consisting of three sexually mature adult generations. The sporocyst is regarded as a 'dendritic' colony. The redia develops as a ciliated 'pro-redia' which metamorphoses into a mature hermaphroditic redia. If the observations of Woodhead are correct, sexual phenomena may be expected to occur also in the larval stages of other trematodes supposedly not too distantly related to the Bucephalidae. La Rue (1926), in his revision of the taxonomy of trematodes having furcocercous cercariae, related the Bucephalidae to the strigeids and schistosomes. Studies on the germinal development of the latter two groups have not thus far tended to support Woodhead's observations concerning the Bucephalidae. In a preliminary note, Woodhead (1932) reported that Leucochloridium, which is not generally regarded as being closely related to the Bucephalidae, also has a polymorphic life-cycle similar to that of Bucephalus. This observation adds considerable support to his earlier conclusions which, if correct, necessitate an entirely different approach from that hitherto taken in interpreting the trematode germinal cycle.

Dollfus (1929, 1932) has recently described cases of sexually mature metacercariae and has postulated that they may be progenetic, that is, retaining the degree of somatic differentiation characteristic of the larva at the time sexual maturity is attained. Dollfus (1932) suggested the possibility that both an abbreviated and a 'normal' life-cycle occur in one of the Lepodermatoidea having sexually mature metacercariae, although a vertebrate host has not been found.

With such a diversity of observations and opinions, it is apparent that the interpretations of trematode germinal development thus far advanced are far from satisfactory when applied to the group as a whole.

III. METHODS.

The larval stages of Cryptocotyle lingua in the liver of the periwinkle, Littorina littorea, were used in the present study. The infected snails were collected by the writer during the summers of 1931 and 1932 at Woods Hole, Massachusetts. They were crushed and the livers having lighter infections were fixed in a number of fluids, including Flemming's strong solution, with and without acetic acid, picro-formol, picro-formol-acetic, Allen's B_3, with and without chromic acid, corrosive sublimate-acetic, and saturated aqueous corrosive sublimate. Flemming's solution with acetic acid and picro-formol-acetic gave the best results, penetration being poor with most of the others employed. The material was imbedded in paraffin and sectioned at six and seven microns, such sections requiring less reconstruction than thinner ones and, at the same time, giving good cytological differentiation. Heidenhain's iron alum-hematoxylin and safranin, both with and without counter stains, were employed, the former with eosin generally giving the better results. Living material was studied by teasing the larvae from the host tissue, compressing slightly with a no. 1 cover-glass on a slide, sealing with vaseline or paraffin to prevent evaporation, and examining with the oil-immersion objective. This method, however, was of little value in the study of germinal development. Fig. 11, Pl. 32, was made with the aid of a camera lucida; all other drawings were made from measurements free-hand and to the same scale. The photomicrographs of Plates 33–5 were made with a Zeiss camera, using Eastman (Wratten & Wainwright) panchromatic plates.

IV. Observations.

The Immature Redia.

The miracidium-mother-sporocyst was not observed during this study although thousands of sections were carefully examined. Very young rediae, however, were found in considerable numbers in material having lighter infections. They were also present in livers having heavier and older infections, usually being located in the lymph spaces just beneath the epithelium of the large bile duct and in interlobular spaces of hitherto uninvaded regions of the liver. Their occurrence along with mature cercariae suggests that either multiple infection takes place or the mother-sporocyst persists until a late stage, producing rediae more or less continuously. If the latter is true, the mother-sporocyst is either so lacking in organization that it cannot be recognized as such, or remains in some part of the host other than the digestive gland where it produces large numbers of rediae which make their way into the liver to complete their development. Since it was expected that at least some of the livers collected would contain sporocyst material, the remainder of the snail was not prepared for study. The youngest rediae observed (fig. 12, Pl. 33) are about 100–25 microns in length and exhibit a low grade of differentiation, the muscular oral sucker and the gut being the only well-defined structures observable in sectioned material. Due to the abundance of connective tissue throughout the posterior half of the larva, the body-cavity is not well defined, but is indistinctly bounded by somewhat heavier strands of tissue which are roughly parallel to the body-wall. Cell boundaries are not visible and the nuclei of the body-wall, supporting tissue of the sucker, and the primordial germ-cells are indistinguishable with respect to size and staining reaction. This is clearly seen in fig. 12, Pl. 33. The germ-cells lying among strands of connective tissue within the region of the future body-cavity are distinguishable from all other cells of the body only by their position. At this stage, the large vesicular and the small densely-staining nuclei, characteristic of the supporting tissue of the sucker in older rediae, are all of the small darkly-staining type. The dense appearance of all the nuclei of the very young redia is only slightly affected by destaining which completely bleaches the surrounding host tissues.

Germinal Origin and Differentiation.

The primordial germ-cells are believed to be distinct from somatic tissues when the young redia leaves the mother-sporocyst, since further cell divisions are not observed until germinal development is initiated. Mitotic figures are of extremely rare occurrence in the somatic cells of the redia at any stage. The nuclei of the primordial germ-cells (fig. 1, Pl. 32) are round or oval in outline and about 3·5 microns in diameter. The centre and periphery of the darkly-staining nucleus are intensely blue-black, indicating a higher chromatin concentration at these parts of the cell. Cytoplasm and cell boundaries are not distinct; the nuclei appear to lie in light areas bounded by strands of connective tissue. Germinal differentiation exhibits a distinct anterior-posterior gradient, the more anterior of the germ-cells initiating the process. This development is characterized by increase in size, progressive nuclear changes, and condensation of cytoplasm with the gradual appearance of more distinct cell boundaries. As the germinal nucleus grows (figs. 2, 3, Pl. 32), the chromatin becomes more diffuse, appearing first as dark masses (fig. 2, Pl. 32) more or less evenly distributed throughout the lighter nucleoplasm. The karyosome appears as an irregular body near the centre of the nucleus. Further differentiation is characterized by continued growth and less intense staining. The chromatin appears as small knots on a reticulum which is denser near the periphery of the nucleus. The karyosome also increases in size, approaching 2 microns in diameter, becomes more spherical in form and is characteristically eccentric in position (figs. 3, 4, Pl. 32; fig. 13, Pl. 33). The cytoplasm becomes well defined and is often concentrated on opposite sides of the nucleus, forming the cytoplasmic nodules described by several workers. This condition is not constant, the cell often being roughly triangular in shape, due to pressure of surrounding cells (fig. 13, Pl. 33). The cytoplasm is best observed in material fixed with Flemming's strong solution without bleaching. The fixative stains the cytoplasm a light brown colour and brings out its granular appearance (fig. 16, Pl. 34) better than any counterstain that was employed. In the full-grown germ-cell (fig. 4, Pl. 32), the nucleus is 7–8 microns in diameter and closely resembles that of the mature oogonia of the adult worm. Fig. 13, Pl. 33, is a photograph of a cross-section through a young redia just behind the gut, showing several of these mature germ-cells. Sections posterior to the one represented in this figure contained varying stages of germinal differentiation as represented in figs. 1, 2, and 3, Pl. 32. As the zone of growth and differentiation of the germ-cells proceeds posteriorly, the fully grown cells may be observed to undergo equal division, resulting in an increase in the number of mature germ-cells which, at a later stage, may form a distinct group (fig. 19, Pl. 34) at the posterior end of the body-cavity.

Cleavage.

By repeated division, the mature germ-cell of the redia forms a cercarial embryo or germ-ball without the intervention of germ-masses. The first cleavages were studied with especial care, since they have been reported by several workers to be maturation divisions. Early divisions are characterized by the appearance of a typical somatic spireme stage (fig. 6, Pl. 32). The spireme appears to be a continuous thread, although this observation is based on reconstructions and the finding of exceptionally long pieces in single sections. In no case has this thread appeared double nor has there been observed any condensations that could be interpreted as syndesis or synizesis. The spireme apparently breaks into segments which go directly into the spindle as twelve chromosomes identical in form with those observed in the dividing cells of the mature worm from the vertebrate host. Spindle fibres and distinct centrioles are seen in favourable material. The first cleavage results in the formation of a smaller micromere and a larger macromere. The micromere, which is shown in fig. 5, Pl. 32, in a stage of condensation with two karyosomes, is the next to divide, resulting in a three-cell stage composed of the macromere and the two micromeres. The micromeres divide next, producing a five-cell embryo, three cells of which are shown in fig. 7, Pl. 32. This figure also shows the chromosomes on the equatorial plate of the macromere which is dividing to form a six-cell germ-ball, composed of four micromeres and two macromeres. It has not been definitely determined which of these macromeres divides next, but one of them apparently does and gives rise to a macromere and the seventh or investing cell (fig. 8, Pl. 32). Both macromeres, as shown with divided karyosomes in fig. 8, Pl. 32, are the next to divide. Fig. 16, Pl. 34, is a photograph of a later stage, showing the investing cell and four cells produced by the division of the two macromeres. The succeeding divisions of the cells of the germ-ball are exceedingly difficult to follow on account of their irregularity, and the characteristic degeneration of many of the nuclei of the germ-ball. Fig. 10, Pl. 32, which is a section of the later embryo, shows one of these degenerating nuclei and one, possibly two, investing cells. After the first cleavage, cell boundaries of the germ-ball disappear (fig. 16, Pl. 34) and the nuclei appear to be imbedded in a common cytoplasmic mass. The chromosomes shown in microphotographs 17*a* and 17*b*, Pl. 34, which are consecutive sections of a dividing macromere in a young germ-ball, are represented also in figs. 9*a* and 9*b*, Pl. 32. The chromosomes are diploid (twelve) in number and exhibit the same characteristic sizes and shapes that were described for the chromosomes of the adult (Cable, 1931). Since they have often been observed in metaphase, the diploid number of chromosomes in the germ-ball stage is unmistakable. Figs. 11 (Pl. 32), 14, 15 (Pl. 33), 16, 19 (Pl. 34), 21 and 22 (Pl. 35), which represent sections of older rediae, show various stages of germ-ball development from the undifferentiated primordial germ-cells to mature cercariae. The embryos towards the anterior end of the rediae are always the more advanced. Between them and the posterior end there is a continuous gradation of development. In younger rediae, undivided and even undifferentiated germ-cells are found clearly distinct from the cells of the wall at the posterior end of the body-cavity. In more mature rediae, which contain mostly advanced cercarial embryos, the movements of these cercariae may change the position of the younger embryos so that the order of development and differentiation is not as apparent as in younger rediae; but even in these cases, the natural arrangement is rarely so changed that it cannot be recognized. The germ-cells at the posterior end retain their position until the last of them has started to divide and the redia becomes a degenerate sac filled with cercariae.

The Relation of the Soma of the Redia to Germ-Cells and Germ-Balls.

The exact relation of the body-wall to the germ-cells of the youngest redia observed (fig. 12, Pl. 33) is difficult to determine, due to the lack of differentiation at this stage. As described above, the wall is not well defined and its cells cannot be distinguished cytologically from the germ-cells contained in the vaguely delimited body-cavity or from the cells of the supporting tissue of the sucker. Nuclei are often observed lying directly on the strands of connective tissue marking the inner limit of the body-wall. It cannot be stated with certainty whether these nuclei are germinal or somatic in nature. As soon as growth and differentiation of the germ-cells are initiated, however, the body-wall becomes well defined (figs. 13, 14, Pl. 33), although cell boundaries remain indistinct. Nuclei are fairly numerous in the thick body-wall of the young redia which contains only a few small germ-balls at the most advanced stages of development (fig. 14, Pl. 33). Posterior to the gut, these nuclei are of the lightly-staining type, except for the occasional occurrence of a small dark nucleus similar to those found in large numbers in the heavy supporting tissue of the sucker. As the redia increases in size, the nuclei of the body-wall appear to become less numerous. This observation is not believed to indicate that the nuclei of the wall enter the body-cavity, becoming germ-cells. Although definite counts have not been made, it seems instead that, due to a lack of multiplication of these nuclei, their number remains fairly constant and, as the redia becomes larger, the nuclei of the body-wall are separated (fig. 22, Pl. 35). When the body-wall becomes greatly expanded, the nuclei are flattened (fig. 21, Pl. 35) much as described for the rediae of several other trematodes. No convincing evidence has been found at any stage that germ-cells arise from the body-wall, although they, as well as germ-balls, may appear in contact with it. This association is believed to be a secondary one.

At approximately the time that germinal development is initiated in the young redia, the cells of the supporting tissue of the sucker become differentiated into the two types mentioned above. The small opaque nuclei appear scattered among the nuclei of the larger type, and also as a distinct cluster (fig. 15, Pl. 33) at the side of the gut. They appear elsewhere in the supporting tissue singly or in small loose clusters (fig. 18, Pl. 34), and one or more may lie very close to a large vesicular nucleus, forming a group which resembles a macromere with micromeres or, due to the dark appearance of the smaller cells, might erroneously be interpreted as an ovum with polar bodies. The large vesicular nuclei with their irregular mass of cytoplasm resemble the parenchymal cells of the adult worm.

Organization and Differentiation of the Cercaria.

Soon after their appearance, the investing cells develop into a distinct membrane completely enclosing the germ-ball. In the more advanced germ-ball, many of these investing cells have become extremely flattened and stain an intense blue-black. The nuclei of the germ-ball retain their germinal appearance for a time; but, as the embryo becomes larger, they decrease in size and are stained more lightly. All stages of this differentiation can be followed continuously from the posterior to the anterior end of the redia. The older germ-balls are characterized by an extremely loose organization (fig. 21, Pl. 35) and, in most preparations, the younger embryos contain distinct and characteristic cavities. Although this condition is enhanced by fixation effects, since it is more apparent in the deeper regions of the infected liver where the fixative did not penetrate properly, it is characteristic of the most favourable material as well. This loose organization is suggestive of dissociation but a careful examination renders this interpretation untenable. There is no evidence that the embryos dissociate completely. The masses of cells which appear to be more or less isolated by the loose organization do not have any features indicating that they may be secondary embryos. As a matter of fact, this dissociation-like appearance is found in embryos possessing well-defined cercarial organ anlagen, the first of which to become differentiated are the large penetration gland-cells and the nuclei of the cells which will form the future excretory bladder. The eye-spots and rudiment of the oral sucker are the next well-defined cercarial structures to appear. Germ-cells, recognizable as such, are first seen at a later stage as a number of small opaque nuclei just anterior to the excretory bladder. It has not been possible to trace them back to an early germ-ball stage. Fig. 20, Pl. 35, shows the genital anlage between the excretory bladder and the penetration glands of a mature cercaria outside of the redia. The anlage shows no evidence of a separation into gonads, and its nuclei resemble those of the primordial germ-cells of the young redia. They do not undergo further differentiation until the metacercarial or early adult stage, in which the anlage is separated into the testes, ovary, and accessory structures.

Degenerating Nuclei and Germ-Balls.

A considerable number of nuclei in each germ-ball seem to degenerate, if their reduced size and intense staining can be taken as an indication of that condition. For convenience in later discussion, these structures are designated as 'degenerating nuclei' although their exact nature is as yet undetermined. These small opaque nuclei, which appear during early cleavage, resemble extranuclear karyosomes (fig. 11, Pl. 32; figs. 16, 19, Pl. 34). They are first recognized by a decrease in size and an accompanying condensation of chromatin (fig. 10, Pl. 32). In later stages of the germ-balls (fig. 22, Pl. 35), they are found in greater numbers and appear in Crytocotyle much as figured by Looss (1892) for Amphistomum subclavatum. They have probably been regarded by some investigators as degenerating polar bodies but it seems that they are too abundant to be interpreted in that manner. Furthermore, entire germ-balls are commonly observed in a state of degeneration (fig. 19, Pl. 34; fig. 22, Pl. 35). These degenerating germ-balls, which were also observed by Looss (1892), Tennent (1906), and possibly others, are usually seen near the posterior end of the redia as a mass of darkly-staining nuclei, varying in size. Macromeres and micromeres can be recognized in some cases and there are often two or more such masses in a redia. These degenerating germ-balls eventually disintegrate and their components are observed scattered among the cercarial embryos where they might easily be mistaken for primordial germ-cells or interpreted as polar bodies by one not familiar with the material. Their degeneration products may serve to nourish the remaining germ-balls. It will be recalled that in the adult (Cable, 1931) degenerating nuclei were observed in the cytoplasm of the oocyte just before fertilization.

V. Discussion.

In considering the evidence concerning germinal lineage in the digenetic trematodes, it is patent that studies dealing only with sporocyst and redial generations can render conclusions that are applicable to those stages alone. The concept of germinal lineage assumes that by the division of a stem-cell early in ontogeny, the soma and germ become separated into two distinct lines. The tissues of the somatic line enclose, nourish, and protect the cells of the germinal line. The early segregation of germ plasm, and its subsequent continuity and cytological distinction from the soma, are well demonstrated in several forms including Ascaris, certain of the coelenterates, and many insects and crustaceans. With the exception of the hydromedusae, the life-cycles of these forms are relatively simple as compared with those of the digenetic trematodes. In the latter group, where three or even more hosts may be necessary for the completion of the life-cycle, the germinal line may not be as distinct throughout the cycle as has been supposed from studies dealing with only the larval stages in the molluscan host. Many investigators, furthermore, have described the origin of germ-cells from the body-wall of sporocysts and rediae, and hence maintain that the germinal line is discontinuous in these stages. Only the definite demonstration of the production of germ-cells from the soma of sporocysts, rediae, and cercariae can disprove the concept of germinal continuity in the Digenea. If the cells of the germinal line are segregated early in ontogeny and remain distinct from the soma throughout the life-cycle, the requirements for germinal lineage are fulfilled regardless of whether polyembryony, parthenogenesis, or hermaphroditism is characteristic of multiplication during larval stages.

Leuckart (1886) suggested that the germ-cells of sporocysts and rediae are derived directly from the fertilized egg of the adult and are merely enclosed by the soma of the larval stages. Kathariner (1904, 1920), Dollfus (1919), and Brooks (1928, 1930) have been the chief advocates of this hypothesis and all have postulated germinal continuity from fertilized egg to fertilized egg, although their studies have not dealt with entire life-cycles. Several investigators including Looss (1892), Tennent (1906), Faust (1917), and Dubois (1929) have described the origin of germ-cells from the body-wall of sporocysts and rediae. These observations oppose the concept of germinal continuity since they indicate that the germ-cells of larval stages are not directly descended from the fertilized ovum. A consideration of the development and differentiation of cercariae also throws doubt on the continuity of the germinal line in that stage of the life-cycle.

In Cryptocotyle, the germ-cells are not recognizable until the germ-balls contain several hundred cells and the penetration glands, excretory vesicle, and the oral sucker have become differentiated. Descriptions of other species of cercariae indicate a great variation in the degree of sexual development of the 'mature' larvae. In a few species, the descriptions definitely state that the genital anlagen were not observed, while a number of workers have not mentioned the germ-cells at all. In view of the fact that the mature cercaria is a highly differentiated larva, it seems that at least recognizable germ-cells would be present if germinal lineage continued through this stage of the life-cycle. In other species of cercariae, more or less well-developed genital anlagen are present in mature specimens. In Cercaria wardi, Cercaria inversa, and Cercaria douthitti, the gonads are represented by a single undeveloped mass of germinal cells. Distinct testicular and ovarian anlagen with lines of nuclei representing the gonoducts are described for Cercaria inhabilis, Cercaria robusta, Cercaria infracaudata, and many others, while Cercaria fusca and Cercaria macrostoma are sexually mature with eggs in the uterus at the time the larvae leave the snail. This great variation in sexual differentiation of mature cercariae may be due to:

1. An earlier segregation of germ-plasm in some species than in others.
2. Longer or shorter periods of development in the mollusc.
3. More rapid germinal development in the case of larvae having well-defined gonads and gravid uteri.

There is evidence for germinal continuity, however, through a part of the life-cycle. In general, the miracidia of trematodes contain indisputable germ-cells at an early stage and a few enclose well-formed rediae at the time of hatching. Since the miracidium metamorphoses into the mother-sporocyst and the germ-cells are undeniably carried over in the body-cavity of the latter stage, germinal lineage must be accepted for that phase of the life-history in many trematodes. This early segregation of germ-cells in the miracidium has been well established for so many species, that, in the absence of direct observation of the miracidium-mother-sporocyst, it is believed that Cryptocotyle lingua is probably no exception to this rule. It cannot be stated at this time whether or not somatic cells of the more mature sporocyst contribute to the reproductive cells of that stage. It is not believed likely, however, in view of observations on germinal development in the redia. The germ-line continues through the redial stage of Cryptocotyle where it is represented by the small primordial germ-cells of the very young redia. As described above, these germinal cells are so similar to all other cells of the young redia that they can be distinguished with certainty only by their position, and even this criterion is difficult to apply in the case of some nuclei that appear to lie very close to the faintly delimited inner boundary of the body-wall. Cells of the wall have never been observed to exhibit any characteristics indicating that they contribute to the germinal line. It is concluded, therefore, that germinal lineage occurs with certainty in the redia and probably also in the sporocyst since it is still nearer the fertilized egg than is the redial generation.

Germinal lineage in Cryptocotyle seems to disappear, however, after the initiation of germ-ball formation since germ-cells are not recognized in the cercarial embryo until a late stage of development. It seems that at least some of the cells of the germ-ball are equipotent or totipotent in the sense that they are not restricted to either somatic or germinal lines, but give rise, at a later stage, to elements of both. It might be argued, on the basis of Brooks' (1930) hypothesis, that germinal precocity, which serves to continue the germinal line in sporocysts and rediae, becomes so decreased in intensity in the germ-ball that somatic factors dominate development. As a result, the germ-plasm becomes inhibited to such an extent that its lineage is interrupted and the germ-cells arise at a later date from equipotent cells that are, until definite segregation, as much somatic as germinal in nature. One would expect an earlier segregation of germinal elements than has been observed in the cercariae of Cryptocotyle and many other species, if germinal continuity is as distinct as Kathariner (1920), Dollfus (1919), and Brooks (1930) have maintained. After the appearance of the genital anlage in the cercaria, the germinal line becomes re-established and continues in a slowly differentiating state through the metacercarial stage. After the parasite becomes established in the final host, germinal development is accelerated and sexual maturity is quickly attained.

It should be stated again that no evidence has been found in the present study that would indicate the occurrence of maturation or other sexual phenomena in the redial stage of Cryptocotyle. According to Brooks (1930), the following may be misinterpreted as polar bodies:

'1. Small darkly-staining cells such as were described by Reuss (1903) as polar bodies but shown by Rossbach (1906) to be of another nature.

'2. Micromeres embedded in cytoplasmic nodules as are described by Faust (1917).

'3. Kåryosomes of cells lying below or above the centre of focus, or belonging to cells in which the remainder of the cell did not take the stain well.

'4. Snail eggs undergoing maturation. This error is very easily made by workers new to the material.

'5. Parts of karyorrhectic nuclei.

'6. Artifacts such as dirt, undissolved stain, &c.'

Other things that may be mistaken for polar bodies are:

1. The components of degenerating germ-balls which have been described by a number of workers and also frequently observed in Cryptocotyle.

2. The association of one or more small dense nuclei with a larger vesicular nucleus in the body-wall and supporting tissue of the sucker. Brooks (1930) has pointed out that the sporocysts of some trematodes have thickened portions of the body-wall which are in reality rudimentary suckers. Such structures with parenchymal cells similar to those of Cryptocotyle could be easily mistaken for ovaries containing maturation stages.

The micromeres of the young germ-balls are the most likely of all structures to be misinterpreted as polar bodies. The writer's study of the chromosomes of Cryptocotyle during early division stages precludes any possibility of reduction. The diploid chromosome number persists from the fertilized ovum of the adult, throughout larval development and until the first maturation division of the oocytes and spermatocytes in the next adult generation. It might be argued that pseudomaturation, which is characteristic of diploid parthenogenesis in such forms as copepods, echinoderms, and certain insects, occurs in the trematodes and that the micromeres are true polar bodies. The absence of any meiotic-like phenomena such as pseudoreduction, synapsis, syndesis, &c., negates the occurrence of pseudomaturation in Cryptocotyle rediae. On account of their number, it cannot be maintained that the degenerating nuclei of the germ-ball are polar bodies. It is concluded, therefore, that no form of maturation occurs in the redial stage of Cryptocotyle lingua.

Considerable emphasis has been placed on the presence or absence of ovaries in sporocysts and rediae by a number of investigators. Since true ova do not occur in the rediae of Cryptocotyle, it is apparent ipso facto that a true ovary would not be present. Germinal multiplication does take place by equal division of mature germinal cells before cleavage begins. In many preparations, a mass of mature cells, none of which had started cleavage, was observed at the posterior end of the redia (fig. 19, Pl. 34). There is no evidence that such a group is derived from a single cell in the manner described by Brooks for other trematodes; the mass cannot therefore be interpreted as a germ-mass in the sense implied by him. The region in which this germinal multiplication by equal division occurs at a given time may be termed the zone of multiplication. Behind this zone in the young rediae, all stages of growth and differentiation of germinal cells were observed. In older rediae, however, the zone of multiplication moves posteriorly until all the germinal cells are finally included in it.

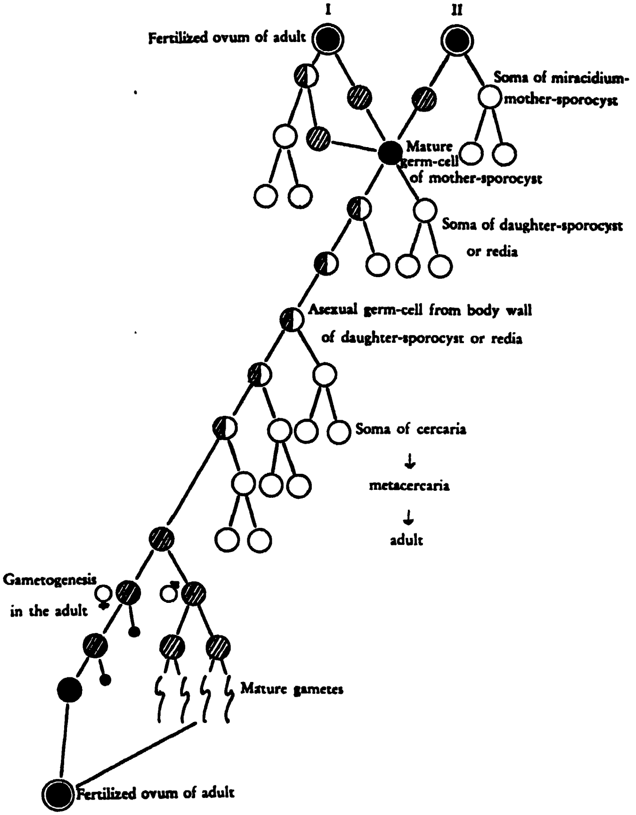
It has already been mentioned that, in the very young redia, cell boundaries are indistinct, the body-cavity is poorly defined and a considerable amount of parenchymal tissue is present. It is believed that the strands of this tissue may play an important part in the interpretation of germinal development. As the first of the germinal cells present in the young rediae become mature and begin to divide, considerable pressure must be exerted on the surrounding cells, and the abundant strands of connective tissue may be pressed forward and backward with the result that the growing and dividing germinal cells appear to be enclosed in a membrane. As development of the enclosed cells ensues, the strands surrounding the group are parted and the contained embryos are freed into the body-cavity. The body-cavity itself seems to become well defined only after germinal development has broken down the obscuring net of connective tissue.

Fig. 22, Pl. 35, is a photograph of a redia showing groups of embryos suggestive of the dissociating germ-masses described by Brooks (1930). A careful study of this and other similar sections revealed that, when present at all, the restraining sheath of connective tissue was found only on the side next to the body-cavity. It was found also that, ranging from the more advanced embryos near the open body cavity, there is a gradation posteriorly to undivided and even undifferentiated germinal cells without a continuous intervening membrane such as would be expected if the group of cells, similar to those seen in fig. 22, Pl. 35, constituted a true germ-mass in the sense of Brooks.

The section represented in this figure is not a longitudinal section through the posterior end of the redia since this part of the larva was bent at a considerable angle to the major portion of the body, the angle at which the section was cut being indicated by the apparent thickness of the body-wall at the end of this section. It is evident, then, that serial sections must be studied with especial care in obtaining an accurate picture of the redia as a whole. Aside from such observations as the one just explained, no phenomena that could possibly be interpreted as germ-mass formation and dissociation have been observed in the rediae of Cryptocotyle. The loose organization of cercarial embryos has been shown in the preceding section to be not a true dissociation but a regularly occurring feature of cercarial development. Although Brooks did not observe germ-masses in some of the species of cercariae studied by him, he concluded that they were formed during larval multiplication, since he did find 'ex-components'. It seems quite possible that these cells may have developed in a more direct manner similar to that observed in Cryptocotyle.

In attempting to determine the significance of the apparent degeneration of germ-balls in the rediae of Cryptocotyle, it was found that a possible explanation may be afforded by the mesozoan, Dicyema. Although the writer has not studied the Mesozoa (Rhombozoa) at first hand, the account of Hartmann (1925) makes it possible to compare certain features of the life-history and germinal development of Dicyema with those of the trematodes. In the young agametic generation of this mesozoan, the centre of the body is occupied by a single, multinucleate axial cell. The nuclei of this cell are of two types, a single primary vegetative nucleus and a number of germinal nuclei, both types arising from division of the primary nucleus of the axial cell. The primary vegetative nucleus divides, giving rise to a secondary vegetative nucleus. The degenerating germ-balls of the trematodes, like the vegetative nuclei of Dicyema, are also derived from cells similar to and very closely associated with the germinal cells which produce normal embryos and, if they have any function at all, it may be that of nourishing the surrounding embryos. These considerations suggest that the degenerating germ-balls may be descendants of a cell in the young redia which corresponds to the vegetative nucleus of Dicyema. Certain observations indicate that this may be the case. In fig. 14, Pl. 33, a longitudinal section of a young redia, two large and two small darkly-staining nuclei are seen between the zone of mature germ-cells and that of the intermediately differentiated germ-cells. Since these nuclei, and also the degenerating germ-balls at a later stage, lie considerably anteriorly to the primordial germ-cells, and are separated from them by a number of intermediate stages of differentiation (in the young redia) and cleavage (in the older redia), it is believed that these nuclei give rise to the embryos which degenerate after reaching a considerable size. It seems quite possible, in view of these observations, that these dense nuclei correspond to the vegetative nuclei of Dicyema. Proof of this relationship is lacking since the writer has not studied Dicyema from actual material, but, if it is true, it affords additional evidence that the Mesozoa are degenerate flatworms and not forms intermediate between the Protozoa and the Metazoa. In the cleavage of both the agamete of Dicyema and the mature germinal cell in the redia of Cryptocotyle, a macromere and a micromere are produced by the first division and the five-cell stage consists of a macromere surrounded by four micromeres. The condensation of cytoplasm around the differentiating germ-cell of Dicyema also has a counterpart in the differentiation of the primordial germinal cells in the redia of Cryptocotyle.

Charts which illustrate in graphic form the various hypotheses of trematode germinal development have been helpful in evaluating the observations of other investigators and interpreting the germ-cell cycle of Cryptocotyle. The great variation in the life-histories and in the details of development, that have been reported, render it impracticable to construct charts showing all of these differences. An attempt has been made, therefore, to combine these variations in four diagrams representing the principal theories of germinal development in the Digenea. These charts are intended not to represent the number of cell divisions, but rather to show only the general features of germinal development and its relation to the soma. Since metamorphosis extending over several generations does not account for germinal multiplication in larval stages, it may be regarded as applicable

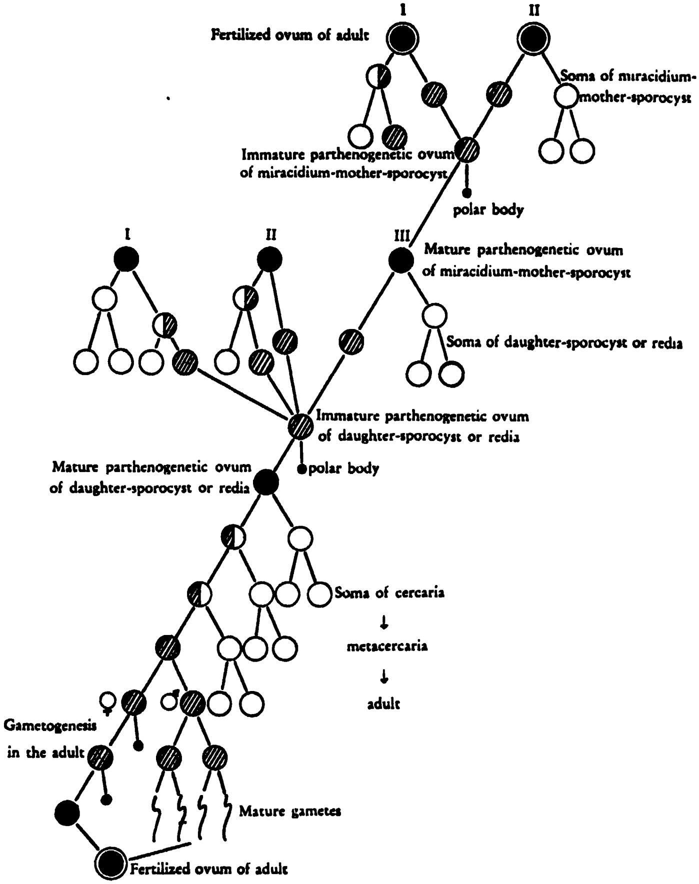

I
II
Fertilized ovum of adult
Soma of miracidium-mother-sporocyst
Mature germ-cell of mother-sporocyst
Soma of daughter-sporocyst or redia
Asexual germ-cell from body wall of daughter-sporocyst or redia
Soma of cercaria
↓
metacercaria
↓
adult
Gametogenesis in the adult
♀
♂
Mature gametes
Fertilized ovum of adult

TEXT-FIG. 1.
Metagenesis.

only to portions of the life-cycle and thus may be applied to any of the four hypotheses discussed.

Text-fig. 1 represents the concept of metagenesis or alternation of generations. This theory implies the alternation of sexual and asexual phases of reproduction and interprets the production of one or more intercalary stages of the trematode life-cycle as a process of internal budding from the wall of the sporocyst or redia. The cells giving rise to daughter-sporocysts or rediae may have a number of possible origins: they may come directly from the fertilized egg as the only source; this direct origin may be supplemented by budding from the wall of the mother-sporocyst; or they may be produced only from cells of the sporocyst wall that remain equipotent for germ and soma until the development of daughter embryos is initiated. The latter possibility is believed unlikely in view of the great mass of evidence indicating early segregation of germ-cells in miracidia. The stage of asexual budding, necessary for true metagenesis, may conceivably be represented, however, in the daughter-sporocyst or redia, in which a number of investigators have reported that cercarial embryos develop from cells budded off from the body-wall. Such a process indicates, as shown in the chart, that the germ-cell of the mother-sporocyst gives rise (1) to cells that are purely somatic in nature and compose the sucker, supporting tissues, &c., of the redia; and (2) to cells that, while somatic in appearance and composing the body-wall, have the potentiality of budding or passing directly into the body-cavity and giving rise to cercariae. This process would involve an interruption of germinal lineage in the daughter-sporocyst or redia. This discontinuity is represented in the chart by half-shaded circles which indicate cells equipotent for germinal and somatic elements. Development of post-redial stages is also represented by equipotent cells whose potency is not restricted until the primordial germ-cells of the cercaria are segregated.

Text-fig. 2 represents the concept of heterogeny which postulates the alternation of parthenogenetic and sexual generations in the trematode life-cycle. The validity of this hyopthesis depends on the demonstration of definite maturation phenomena in the germ-cells giving rise to daughter-sporocysts, rediae, and cercariae, since it must be shown conclusively that these cells are true ova. It should be pointed out that the concept of heterogeny does not necessarily imply discontinuity of the germinal line, since maturation phenomena could be associated with reproductive cells that are segregated early in ontogeny. Most of the advocates of this hypothesis, however, have reported that parthenogenetic ova are somatic in origin or at least are derived from the body-wall. A definite ovary has been described in some species. Three possible origins of parthenogenetic ova in the redia are represented in the chart:


I
II
Fertilized ovum of adult
Soma of miracidium-mother-sporocyst
Immature parthenogenetic ovum of miracidium-mother-sporocyst
polar body
I
II
III
Mature parthenogenetic ovum of miracidium-mother-sporocyst
Soma of daughter-sporocyst or redia
Immature parthenogenetic ovum of daughter-sporocyst or redia
Mature parthenogenetic ovum of daughter-sporocyst or redia
polar body
Soma of cercaria
↓
metacercaria
↓
adult
♀
♂
Gametogenesis in the adult
Mature gametes
Fertilized ovum of adult

TEXT-FIG. 2.
Heterogeny.

I. From cells of the body-wall. Described by Tennent (1906) and Faust (1917) for certain species. This mode of origin invalidates germinal lineage.

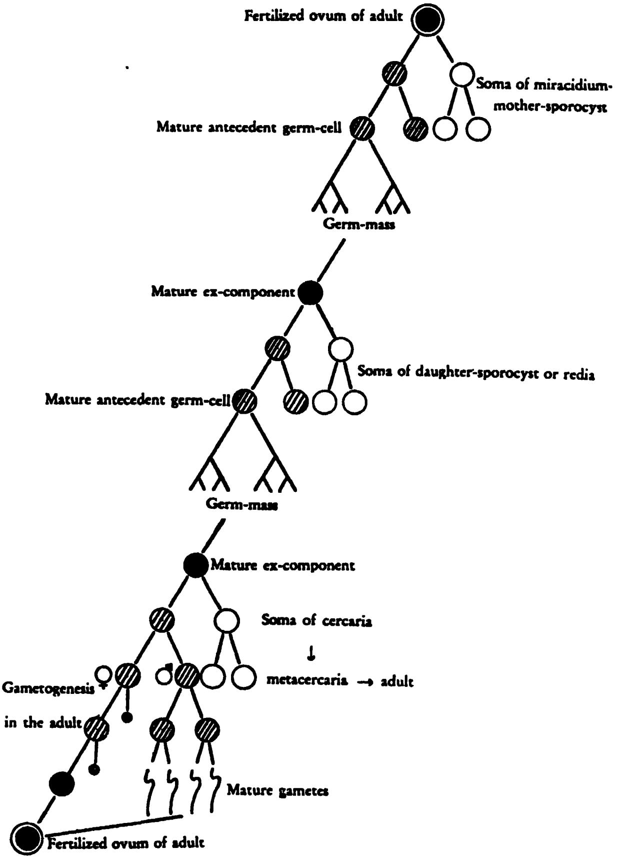
II. Segregation of germ-cells in the young larvae and also production of parthenogenetic ova from the body-wall. Implied by Cary (1909).

III. Early segregation of germ-cells alone. Not definitely postulated by any advocates of heterogeny. Possibly true of the 'ovaries' and 'specialized germinal masses' described by Haswell (1903) and Faust (1917).

In Text-fig. 2, each parthenogenetic ovum is represented as producing a single polar body; as many as three have been reported.

Brooks' (1930) hypothesis of germinal lineage with polyembryony is represented in Text-fig. 3. Germinal lineage is continuous from fertilized ovum to fertilized ovum and the cells of the germinal line are regarded as merely enclosed by the soma of the sporocyst and the redial stages which are themselves sterile. Brooks interprets as polyembryony the formation and dissociation of primary, and perhaps secondary and tertiary germ-masses whose components give rise to the next stage of the life-cycle. In this text-figure, a single germ-mass is represented for each generation involved in larval multiplication. It has been mentioned in the discussion of germinal continuity of Cryptocotyle that continued lineage through post-redial stages, as postulated by Kathariner (1920), Dollfus (1919), and Brooks (1930), is not supported by observations that may be regarded as conclusive in nature.

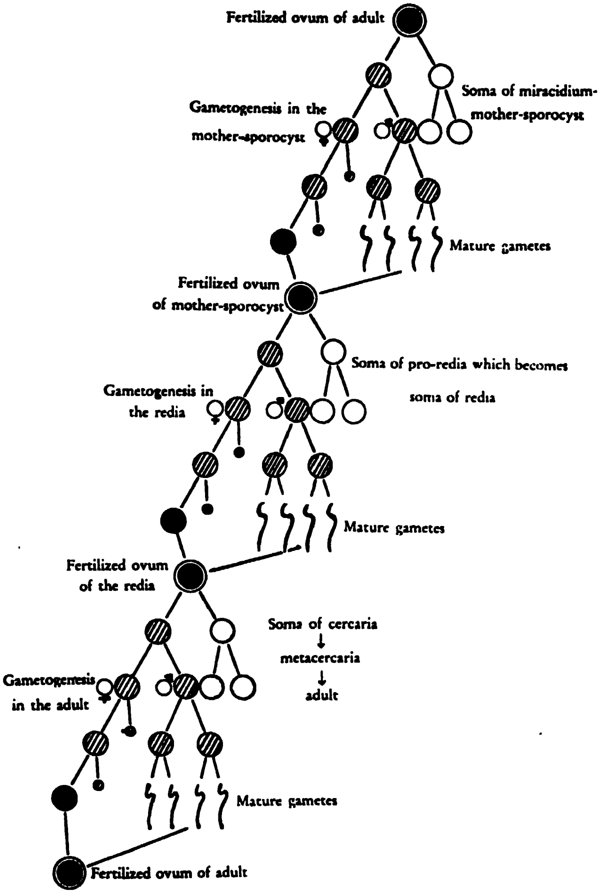
Text-fig. 4 represents Woodhead's interpretation of the germinal line in Bucephalus as polymorphism with three sexually mature hermaphroditic generations. Although Woodhead did not postulate germinal continuity by early segregation of germ-cells in the various stages, for the sake of simplicity the writer has taken the liberty to represent this condition in the chart. The fertilized ovum of the final adult gives rise to a branching sporocyst or 'dendritic colony' whose several tips possess functional ovaries and testes. Gametogenesis and fertilization occur in this stage in a manner similar to that in the final adult worm. The fertilized ovum of the sporocyst gives rise to a ciliated pro-redia which metamorphoses into an hermaphroditic redia also possessing functional gonads. The fertilized ova of the redia produce cercariae which develop into the fina hermaphroditic adults. In a recent note (1932), Woodhead has reported that Leucochloridium has a germinal cycle



TEXT-FIG. 3.
Germinal Lineage with Polyembryony.

similar to that of Bucephalus. This observation makes it impossible to regard polymorphism as restricted to the Bucephalidae, although this group is a peculiar one in many respects and may not be closely related to other digenetic trematodes.

The impossibility of explaining Woodhead's observations on Bucephalus and Leucochloridium on the basis of


Fertilized ovum of adult
Soma of miracidium-mother-sporocyst
Gametogenesis in the mother-sporocyst
Mature gametes
Fertilized ovum of mother-sporocyst
Soma of pro-redia which becomes soma of redia
Gametogenesis in the redia
Mature gametes
Fertilized ovum of the redia
Soma of cercaria ↓ metacercaria ↓ adult
Gametogenesis in the adult
Mature gametes
Fertilized ovum of adult

TEXT-FIG. 4.

Polymorphism with Three Sexually Mature Hermaphroditic Generations.

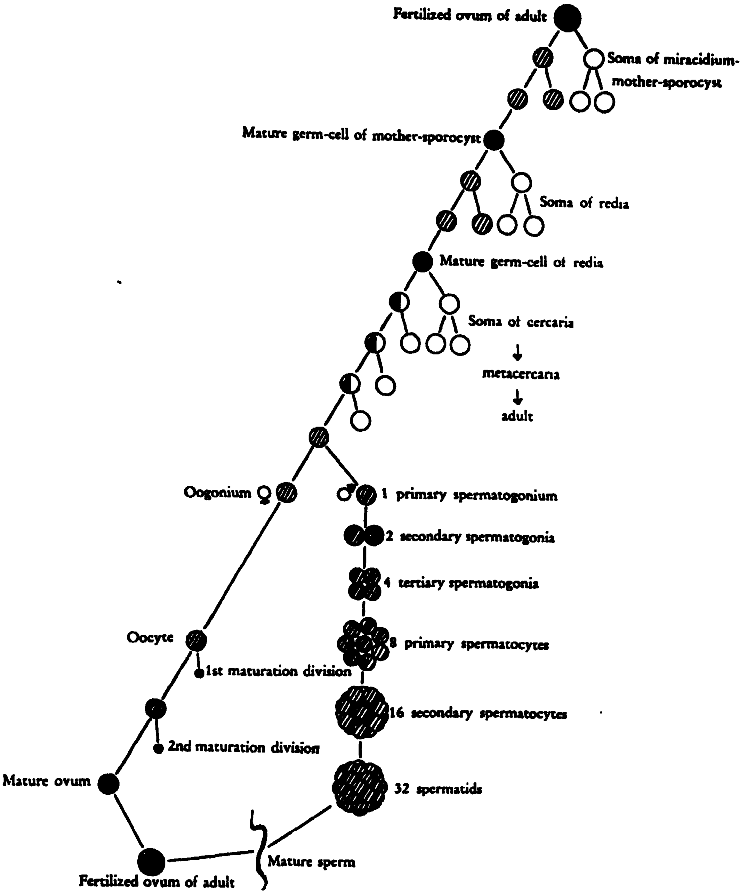
Brooks' concept of polyembryony makes it necessary to approach the problem of trematode germ-cell cycles from an altogether different angle. It appears certain that the only satisfactory interpretation must be based primarily on the phylogeny of the Digenea. It must take into consideration all well-substantiated findings concerning germinal development in the members of this group. It must regard the Digenea as descendants of free-living ancestors and must determine the manner and extent of modification of the germ-cell cycle as a result of the parasitic habit. Although evidence is as yet extremely fragmentary and there is considerable disagreement in the observations and interpretations of different workers, sufficient data are available to suggest a phylogenetic hypothesis of trematode germinal cycles. Woodhead's observations support the view that all digenetic trematodes were primitively parasites of molluscs, becoming sexually mature and completing the life-cycle in those hosts. The two genera, Bucephalus and Leucochloridium, in which Woodhead has reported hermaphroditic stages in the mollusc, are quite dissimilar. It is of interest to note that in both, until quite recently, cercariae have been regarded as arising directly from the mother-sporocyst. The redia of Bucephalus, according to Woodhead (1931) is inconspicuous, and its primitive nature is indicated by the ciliated covering of the pro-redial stage. Bucephalus and Leucochloridium have the minimum number of intercalary stages in the life-cycle and, if sexual maturity characterized the primitive trematode in the mollusc, these forms may be regarded as having been changed to a less extent by parasitism than most other trematodes. A ciliated pro-redia, such as that described by Woodhead for Bucephalus, has not been reported for any other trematode, unless Sewell (1922) was mistaken in his observations on Cercaria indicae XV in which he noted that the sporocysts gave rise to both cercarial embryos and miracidia. These miracidia resemble the pro-rediae of Bucephalus in that they are encapsulated, ciliated, and arise from sporocysts. If Sewell's interpretation is correct, it affords additional evidence that the primitive trematode life-cycle was direct and completed in the mollusc. The occurrence of sexually mature metacercariae, as described by Dollfus (1929, 1932), however, probably cannot be interpreted as an intermediate stage in the evolution of the digenetic life-cycle. It is explained perhaps more satisfactorily by Dollfus as progenesis with extreme sexual precocity and the possibility of both an abbreviated and an extended life-cycle.

Brooks' hypothesis argues for a sudden change in the life-cycle from sexual reproduction to polyembryony on the adoption of parasitism. His concept is an exceptionally fortunate one in many respects since it can explain the presence or absence of germ-masses and any number of intercalary stages. This hypothesis could be easily applied to Cryptocotyle by assuming that germinal precocity is less marked in this species than in those studied by Brooks, and that the germ-cells, instead of forming germ-masses, have a 'normal' tendency to produce larvae directly. Woodhead's findings, however, cannot be explained on the basis of Brooks' hypothesis, adaptive as it is.

It is believed, therefore, that changes due to parasitism have appeared in a gradual rather than a sudden manner, and that the primitive trematode became sexually mature in the mollusc and completed the life-cycle in that host. With the evolution of parasitism, sexual reproduction in the mollusc has gradually disappeared and accessory stages have been acquired. If this is true, it would not be surprising, but indeed expected, that only the male reproductive processes have been lost in some of the present trematodes and their development is essentially parthenogenetic. In perhaps the majority of digenetic trematodes, the life-cycle has been modified to a still greater extent with the result that sexual phenomena in the molluscan host have entirely disappeared and polyembryony, with or without distinct germ-masses, is operative in larval multiplication. The life-cycle in the mollusc may thus have been considerably modified before the vertebrate host was parasitized. After the appearance of vertebrates, the trematodes became established in that group for some reason as yet unknown. The inclusion of the vertebrate host in the life-cycle may have been incidental to its feeding on infected molluscs; or it may possibly have been the result of changes in the trematode as a result of parasitism of the mollusc such that further development in another host became necessary for survival. The concept of gradual change is admittedly hypothetical because of insufficient information, but it seems on the whole more reasonable and applicable to all the facts than the hypothesis that adoption of the endoparasitic habit resulted in sudden changes in germinal development similar to those that have been induced experimentally in other forms by such exogenous factors as lowered oxygen tension, temperature effects, &c. It cannot be denied that such factors have modified the life-cycle in the trematodes, but, in view of the findings of various workers, it seems unlikely that they have induced sudden intercalations of polyembryonic phases in the germ-cell cycle.

The germ-cell cycle of Cryptocotyle is represented in Text-fig. 5. It is concluded that this species is intermediate in position in the proposed phylogenetic scheme of germinal development, since the cercariae develop directly without the intervention of germ-masses in the sense of Brooks, since maturation or other sexual phenomena are lacking in the redia, and since the life-cycle does not have daughter-sporocysts or daughter-rediae. Patterson (1927) defines polyembryony as the production of two or more individuals 'from a single fertilized egg during the course of its early development'. An examination of the literature shows that this phenomenon is extremely variable in nature. In some forms, the first divisions of the fertilized egg may definitely separate and initiate the development of two or more embryos, while in one notable case, that of the armadillo, the fertilized ovum gives rise to a relatively undifferentiated structure, the blastocyst, which shows no indication of producing four instead of a single young until it reaches a considerable size. On account of such cases of delayed embryo formation, a definite distinction between metagenesis and polyembryony does not exist. Stockard (1921), indeed, has pointed out that the embryonic mass, giving rise by budding to a number of embryos, may be regarded as the sexually produced generation. Reproduction in this generation by budding may therefore be regarded as asexual in nature and the life-cycle may be interpreted as metagenetic.

In view of the early segregation of germinal cells in the rediae of Cryptocotyle, and since the cells of this line multiply by equal division, larval reproduction in this trematode is interpreted as a type of polyembryony. The apparent interruption of germinal lineage in the germ-ball, as represented in Text-fig. 5, is coincident with the development of such specialized cercarial structures as the tail and larval glands which are necessary for



TEXT-FIG. 5.
The Germ-Cell Cycle of Cryptocotyle lingua.

establishing infection in the fish host. Concomitant with this somatic differentiation, germinal development is repressed and the genital anlage does not become apparent until a rather late stage. After the parasite becomes established in the final host, germinal development proceeds rapidly and gametogenesis, fertilization, and cleavage follow as represented in the chart and previously described in detail (Cable, 1931).

A consideration of the diverse life-histories and of the phylogenetic affinities of the Digenea, in addition to specific investigations of germinal development that have been made, suggests that any single type of germ-cell cycle may not characterize all members of the order. The writer, therefore, proposes the above phylogenetic concept as a basis for further study rather than a compromise between existing divergent theories. Before an understanding of germinal development in the Digenea as a group will be possible, more data must be secured from a large number of complete life-histories in which the germ-cell cycle is followed from adult to adult. As more life-histories become known, a comparative study will be made possible, and many of the problems related to the origin of complicated life-histories in the Digenea will be better understood.

VI. SUMMARY.

The writer's earlier study (Cable, 1931) on the germ-cell cycle in the adult stage of Cryptocotyle lingua is supplemented by an investigation of germinal development in the larval stages occurring in the marine snail, Littorina littorea. The miracidium-mother-sporocyst was not found although very young rediae were abundant in the material studied. The primordial germ-cells of the young redia are observed in an undifferentiated condition in the body-cavity, which is not well defined due to an abundance of connective tissue. After a period of differentiation, including growth, progressive nuclear changes, and condensation of cytoplasm, the germ-cells multiply by equal division, a process which is interpreted as polyembryony. Germinal differentiation exhibits a distinct anterior-posterior gradient. The mature germinal cells give rise directly to cercarial embryos without germ-mass formation and dissociation or any maturation processes. Although germinal lineage may be traced in the redia, it seems to be interrupted in the cercaria, due to delayed segregation of germ-cells. The soma of the redia does not produce germ-cells at any stage. Evidence is afforded by this and other studies that germinal lineage with sudden intercalations of polyembryonic stages (germ-masses) cannot explain the germinal cycle of the trematodes as a group. In an alternative hypothesis, based on the phylogeny of the Digenea, it is suggested that the ancestors of this group became sexually mature in the mollusc and completed the cycle in that host, possibly before the appearance of vertebrates; and that, with the evolution of the trematodes, sexual phenomena have gradually been lost, while accessory stages and new hosts have been included in the life-cycle. Cryptocotyle lingua is assumed to have been modified to an intermediate extent since sexual reproduction, germ-masses, and the maximum number of intercalary stages are lacking in the larval generations.

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DESCRIPTION OF PLATES 32 TO 35.

LETTERING.

ct, connective tissue; *dgb*, degenerating germ-ball; *dn*, degenerating nucleus of germ-ball; *ga*, genital anlage; *gb*, germ-ball; *gc*, germ-cell; *gt*, gut; *ic*, investing cell; *igc*, immature germ-cell; *k*, karyosome; *mac*, macromere; *mgc*, mature germ-cell; *mic*, micromere; *pgc*, primordial germ-cell; *s*, sucker.

PLATE 32.

Fig. 1.—Primordial germ-cell of the young redia (× 2,466).

Figs. 2, 3, and 4.—Progressive stages in differentiation of germ-cells of the young redia (× 2,466).

Fig. 5.—Two-cell stage of the germ-ball (× 2,466).

Fig. 6.—Spireme stage typical of cell divisions in the germ-ball (× 2,466).

Fig. 7.—Three cells of the five-cell embryo showing division of the macromere to form the six-cell stage (× 2,466).

Fig. 8.—Seven-cell stage with investing cell (× 2,466).

Fig. 9 *a* and *b*.—Twelve chromosomes in the dividing macromere of a young germ-ball as observed in two consecutive sections and photographed in fig. 17 *a* and *b* (× 2,466).

Fig. 10.—Section of a later stage showing the investing cell and a degenerating nucleus (× 2,466).

Fig. 11.—Camera lucida drawing of a young redia showing various stages of germ-ball development (× 466).

PLATE 33.

Fig. 12.—Longitudinal section of a very young redia (microphotograph, × 575).

Fig. 13.—Cross-section at a level immediately posterior to the gut of a young redia showing mature germinal cells with typically eccentric karyosomes (microphotograph, × 1,000).

Figs. 14 and 15.—Longitudinal sections of young rediae showing differentiating germ-cells and young germ-balls (microphotographs, × 500 and × 600 respectively).

PLATE 34.

Fig. 16.—Section through the posterior end of a redia showing germ-balls with investing cells and degenerating nuclei (microphotograph, × 1,100).

Fig. 17 *a* and *b*.—Consecutive sections of a young germ-ball showing the chromosomes of a dividing macromere (microphotograph, × 1,250).

Fig. 18.—Cross-section through the anterior end of a mature redia showing the oral sucker and the nuclei of its supporting tissue (microphotograph, × 750).

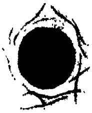
Fig. 19.—Longitudinal section through the posterior end of an older redia (microphotograph, × 900).

PLATE 35.

Fig. 20.—Frontal section of a mature cercaria (microphotograph, × 625).

Fig. 21.—Longitudinal section through an older redia showing undivided germ-cells at the extreme posterior end and the loose organization of cercarial embryos (microphotograph, × 455).

Fig. 22.—Section through the posterior end of a redia showing various division stages and a degenerating germ-ball (microphotograph, × 500).



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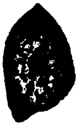

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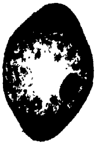

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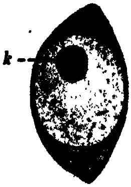

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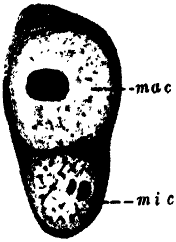

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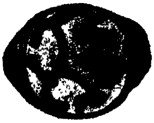

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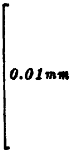

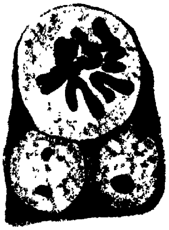

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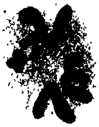

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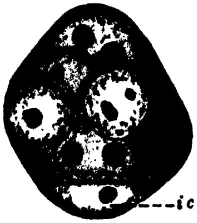

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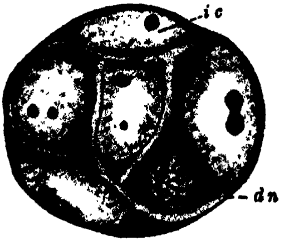

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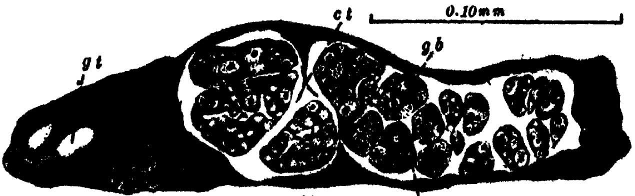

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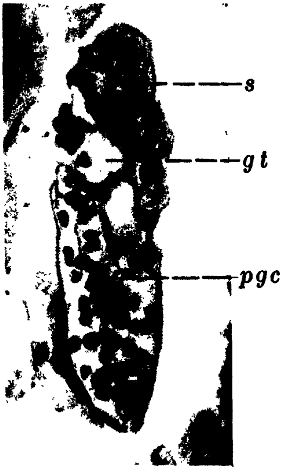

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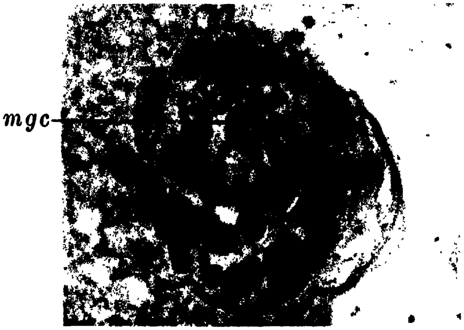

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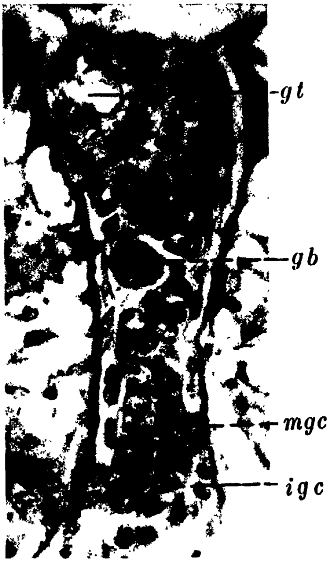

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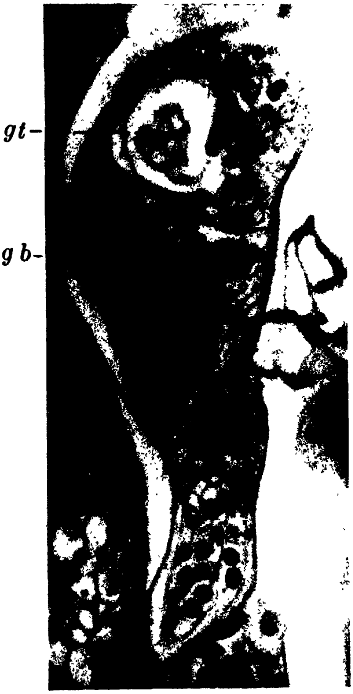

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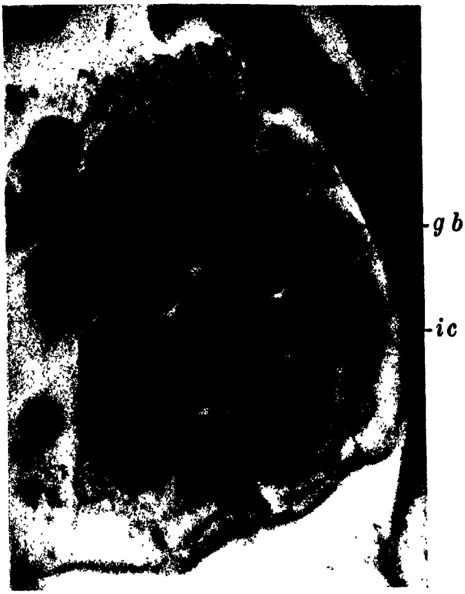

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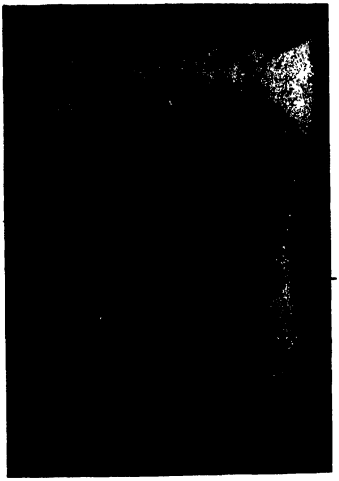

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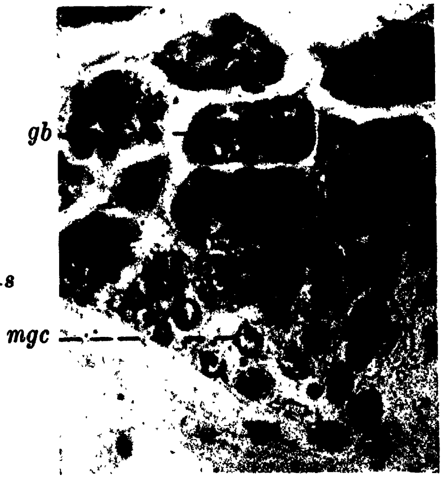

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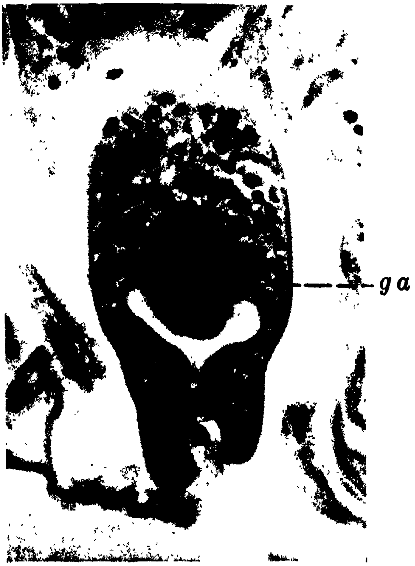

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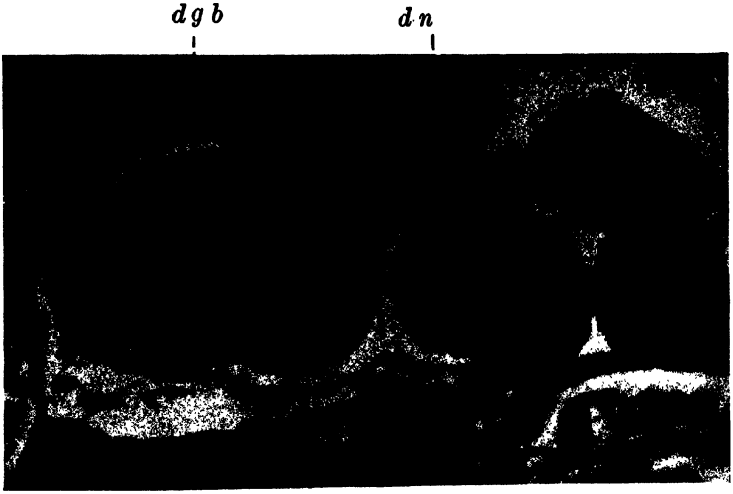

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R. M. Cable, del.

The Golgi Apparatus in the Thyroid Gland of Amphibians, in its relation to Excretion Polarity.

By

Eduard Uhlenhuth,

University of Maryland, Medical School.

With Plate 36.

In 1923 Nassonov reported observations which suggested that the Golgi apparatus is concerned in the elaboration of the secretion droplets of gland-cells; he therefore concluded that the Golgi apparatus is the secretory organ of the cell.

As Bowen (1929) has repeatedly and correctly emphasized, it is necessary to distinguish carefully, in the activity of gland-cells, between two processes, between secretion and excretion. The former designates only that part of the activity of the cell which ultimately leads to the manufacture of the secretion products, while the latter relates to the removal of the secretion products from the cell. Even assuming that the Golgi apparatus plays an essential role in the manufacture of the secretion, such a relation cannot, without further evidence, be considered as indicating that excretion likewise is controlled by the Golgi apparatus.

Those who support their claims for a participation of the Golgi apparatus in the excretion of the secretory products from the cell by referring to Nassonov's work do not fully appreciate the bearing of Nassonov's discoveries. This author has not only not demonstrated a relation between Golgi apparatus and excretion, but has emphasized specifically that the mechanism of excretion of the secretory products is not dependent on the Golgi apparatus (1923, p. 179). In his first article (1923) he states, p. 179: 'Der Mechanismus der Ausführung des Sekretes aus dem Zellkörper . . . hängt augenscheinlich nicht vom Binnennetz ab.' Concerning the special problem of the excretion polarity Nassonov made reference to this in only one place of his first article (1923, p. 161). He says: 'Diese Orientierung des Binnennetzes stets in der Richtung zur Mündung des Sekretionsstromes hin ist für mich besonders interessant, da sie einen weiteren indirekten Beweis für die Beteiligung des Binnennetzes an der Sekretion liefert.' In none of his later articles, however, has Nassonov made any further reference to this problem.

Going through the literature, in an attempt to utilize the position of the Golgi apparatus, at either the apical or basal cell-pole, in the interpretation of the secretion physiology of the cell, one is struck by the complete absence of any experimental data or any other well-founded evidence which would prove that the Golgi apparatus migrates from one pole to the opposite when the excretion polarity so changes, or that the excretion polarity of a cell is determined by the position of the Golgi apparatus. Nevertheless attempts are not wanting of using the position of the Golgi apparatus as an indicator of the excretion polarity.

Courrier and Reiss (1922) seem to be the first ones who, without presenting any evidence to substantiate their conclusions, infer that, in the parathyroids of young cats, the pole at which the Golgi apparatus is located is that at which excretion takes place. In a subsequent article Reiss (1922) employs the same criteria in the case of the hypophysis.

That the position of the Golgi apparatus may sometimes be reversed was noticed by the earlier students of the Golgi apparatus, including its discoverer. Great caution, however, was observed by them in formulating an interpretation. Golgi, in 1909, studied the reticular apparatus in the mucous cells of the gastric mucosa and found a characteristic reversal of the position of the apparatus; he attributed it, tentatively, to the action of internal mechanical forces. At any rate it was obvious, even at that early date, that the Golgi apparatus could hardly have anything to do with secretion polarity, since the cells in question, so far as known, excrete always through the same pole.

In 1926, Bowen for the first time warned against premature conclusions regarding the position of the Golgi apparatus, and showed that in the light of comparative studies there is little ground for assuming that the position of the Golgi apparatus corresponds to the excretion polarity of the cell. He found that the Golgi apparatus in the cells of the epididymis and vas deferens of the cat may have either an apical or a basal position, although the cells of both of these organs excrete only in an apical direction. Referring to the role of the Golgi apparatus in the secretory activity of the cell, he says (p. 409): '. . . it is clear that the reversal is not associated with any particular functional phase of the cell, for all stages in the secretory cycle are found with reversed Golgi apparatus.' And in relation to the excretory polarity he expresses himself in the following terms: 'I have never seen anything which suggests that the reversal of polarity as measured by the position of the Golgi apparatus has any relation to the direction in which the secretory products are extruded' (p. 409).

Similar observations have been made in many other cells (Bowen's article of 1926 contains a brief review of the literature) which so far have not been suspected of excreting through the basal pole. Among the articles pertaining to this matter may be mentioned one by Giroud (1928). This investigator studied the cells in the intestinal wall and in the kidney tubules of amphibians; in the latter cell-type the position of the apparatus seems to vary with the species. Giroud attempts to explain the reversal of the position of the Golgi apparatus in the cells of the two organs studied by him, in terms of pressure changes within the cell. It has been mentioned above that Golgi too suggested mechanical forces as the cause of a reversed position of the apparatus. Bowen (1926), however, considers the role of mechanical forces, in the migration of the Golgi apparatus, as doubtful, at least in the cells of the epididymis (p. 409).

Weatherford, in a recent article (1929), reports investigations of the Golgi apparatus in the cells of the mammary gland. The cells of this organ, according to our present knowledge, excrete always at the apical pole. Nevertheless, the Golgi apparatus, during certain secretory phases, migrates away from its strictly apical position and comes to lie, in part, at the basal cell-pole.

THE GOLGI APPARATUS IN THE THYROID GLAND.

The Golgi apparatus in the epithelial cells of the thyroid gland has been described by a considerable number of investigators.

It should be mentioned, however, that heretofore the thyroid gland was not a suitable object for the study of the physiological role of the Golgi apparatus, since it has been only recently that a fairly correct picture of the secreting thyroid cell has become known. But even at present the structures of the thyroid cell are still under discussion; the physiological interpretation of the cytology of the thyroid is still in a stage in which it needs to draw upon other well-established facts for analysis rather than to be used as supporting evidence in the investigation of other unsettled problems. In particular the direction of the secretory current at different stages of the secretion process is still a much-debated question; it would be unwise to apply any of the current hypotheses relating to the excretion polarity of the thyroid cell in the formulation of a theory postulating a relation between the excretion polarity of the cell and the position of the Golgi apparatus.

Cowdry (1922) was probably the first to study the position of the Golgi apparatus in relation to the excretion polarity of the thyroid cell. He found that in the thyroid of the guinea-pig, in 1 cell out of every 500, the Golgi apparatus lies at the basal side of the nucleus. On the strength of this observation he claimed that the thyroid cells, under certain conditions, may reverse the direction of their excreting activity and excrete through the basal pole, but failed to offer any evidence showing that there exists a relation between the position of the Golgi apparatus and the excretion polarity of the cells. His article is nevertheless often quoted as if he had presented evidence proving that the position of the Golgi apparatus is reversed whenever the secretion polarity of the cell is reversed.

Bowen, in his article referred to above (1926), takes a very definite stand with regard to Cowdry's work. He says (p. 412): 'Cowdry's statement that "Apparently in the thryoid gland alone is there variability in the position of the reticular material, and in the thyroid gland alone have we clear evidence of physiologic reversal in the direction of secretion" is thus hardly in accord with the facts.... I think it unsafe to use this particular case as evidence pro or con for any theory of secretory activities.'

More recently Ludford and Cramer (1929) investigated the position of the Golgi apparatus in the cells of the thyroid gland. In the thyroid of rats, the activity of which was raised to a maximum by exposing the animals to low temperature, the Golgi apparatus was always found at the apical pole. From this observation the authors unfortunately concluded that a normal thyroid, even when activated, excretes always through the apical pole, into the lumen. In exophthalmic goitre glands, especially of one white mouse, the Golgi apparatus in a number of cells occupied a basal position; Ludford and Cramer take this as an indication that in this pathological condition the excretion polarity of the cells is reversed. Far-reaching conclusions upon the causes of the toxic effects of the thyroid hormone in exophthalmic goitre are based upon this supposed difference between the excretion polarities of normal and exophthalmic goitre thyroids. It will be shown presently that these conclusions are premature, as the interpretation made by Ludford and Cramer is obviously incorrect.

In 1926 Schwartzbach and the author of this article (**34** and **35**) found that a substance can be extracted from the prehypophysis of cattle, which possesses the specific property of activating the thyroid cells to a maximum degree, an observation which has since been verified in further studies (see under Schwartzbach, Figge, Uhlenhuth, Zurawski) made in our laboratory and has been confirmed by several other investigators (Paal, 1931; Adams, 1932; Loeb, 1929; Aron, 1930). Similar results may be obtained by grafting the anterior lobe (Ingram, 1928; Grant, 1930). Owing to this technique it is possible to study the secretion mechanism of the thyroid cell with an increased degree of accuracy. At present new concepts of the secretory activity of the thyroid are in progress of formation. Some of the most important problems centre around the question, whether the thyroid cells are capable of excreting not only through the apical, but also through the basal pole. Madeleine Grant (1930), on the basis of indirect evidence, has declared herself in favour of a bipolar excretion mechanism. Zurawski and Uhlenhuth (1932), at a meeting of the American Anatomical Association, demonstrated histological slides of the thyroid of the Californian newt (Tirturus torosus), stimulated by injections of thyroid activator, which lend themselves to the interpretation that during the process of colloid release (from the follicle into the blood sinusoids) the thyroid cells excrete the liquefied cell-content through the basal pole. Nevertheless it appears to the writer of this article that in spite of its highly suggestive character this evidence may not fully satisfy a critical student of the secretion process of the thyroid, and needs confirmation by further work in progress in our laboratory. Yet the interpretation of the facts to be reported presently hinges entirely on the question of the secretory polarity peculiar to the thyroid cell in its different secretory phases. For this reason it is necessary to state that, while the lack of a reversal of the position of the Golgi apparatus in the thyroid cell to be shown in this article is a valuable observation, the interpretation given it here may be only of a temporary nature; its correctness depends entirely on the correctness of the interpretation which we have given to our histological pictures suggestive of basal excretion.

The investigators whose work will be reviewed in the following paragraphs hardly concerned themselves with an analysis of the excretion polarity of the thyroid cell. For this reason the interpretation of their observations will have to be weighed with an even greater measure of caution.

Ingram (1928), using the grafting technique, was the first to show that in the thyroid stimulated by the thyroid activator the Golgi apparatus takes invariably an apical position. His work is open to criticism, however, as he did not follow the development of the secretion processes in chronologically arranged series, nor did he state that, at the time of the death of the animals, there were signs of basal excretion; his illustrations do not convey the impression that the cells were really active.

More recently Wagschal (1931) in Max Aron's laboratory, using our technique of stimulating the thyroid by the thyroid activator, found that in guinea-pigs, too, the Golgi apparatus is located invariably at the apex of the thyroid cells. The cells illustrated in his article do not, however, appear to be highly activated. Moreover, neither in Ingram's nor in Wagschal's illustrations does the Golgi apparatus present the structure which it does have, according to the work described here, in really active cells.

At a meeting of the American Anatomical Association (1932) the writer discussed and demonstrated preparations of the thyroid of amphibians, stimulated to a high degree of function by injections of thyroid activator, and impregnated by Nassonov's osmic acid technique. In no case was the Golgi apparatus found to occupy a basal position. At the same meeting Severinghaus (1932), in discussing his work on the histological differentiation of the cells of the hypophysis, mentioned that he had made similar observations in the thyroids of warm-blooded animals, activated by the same technique.

In this article the results presented in the preliminary paper will be discussed in greater detail and data serving as evidence in support of the conclusions will be submitted.

MATERIAL AND METHODS.

The adult California newt, Triturus torosus, was used in the experiments.

As in previous experiments the anterior-lobe powder was prepared in our own laboratory. The hypophyses were collected by ourselves (in the slaughterhouse), the anterior lobes were separated from the posterior lobes in not more than 40 minutes after the death of the steers, taken home, cut with scissors into small pieces, dried by an electric fan, ground to powder and desiccated in the $CaCl_2$ desiccator.

For injection the powder was extracted, by boiling slightly, in an 0·5 per cent. solution of glacial acetic acid in a 30 per cent. frog Ringer solution; the infusion was filtered and the filtrate neutralized against lithmus paper by a N/5 NaOH solution to a slightly acid end-point. After neutralization 0·5 c.cm. of the liquid contained the extract of 100 mg. dried powder.

The injections were made every other day, into the peritoneal cavity. 0·5 c.cm. per animal was used at one injection. The animals were always killed one day after the last injection.

The thyroids were removed in chloretone anesthesia, under

30 per cent. frog Ringer and dropped immediately into the fixative.

For fixation two different methods were employed. In each case the left thyroid was fixed in Zenker-glacial (without Na_2SO_4); the right gland was fixed and impregnated with osmic acid after the Kolatchev technique as modified by Nassonov. For impregnation the thyroids were left in the osmic acid for two days, at approximately 35° C. Ingram (1928) had found two days just the right length of time to get satisfactory impregnation in the thyroids of tadpoles; the same period of immersion gave very excellent pictures of the reticular apparatus in the salamander thyroid. For embedding paraffin was used. The sections were cut at 7 microns.

THE GOLGI APPARATUS OF THE NORMAL AMPHIBIAN THYROID, IN THE RESTING CONDITION.

In a recent article Charipper (1930) published a study of the Golgi apparatus of the thyroid cells of the perennibranchiate salamander Necturus maculosus. In young specimens he found the reticular apparatus to consist of a number of isolated osmiophilic bodies, while later in the life of the individual it has the shape of a network located at the apical side of the nucleus. Apparently the Golgi apparatus of the amphibian thyroid shows the same changes during the development of the individual and of its organs as have been described by Da Fano (1922) for the Golgi apparatus of the mammary gland of mammals. A similar transformation from a stage of isolated pieces into a network is known to take place during cleavage of the fertilized egg and during development of nerve-cells (for a review of the literature see Jacobs, 1927). A feature characteristic apparently for Necturus was described by Charipper; the reticulum sends a long filament to the apical surface of the cell where it ends in a group of granules.

While the species used by Charipper is one which not only does not metamorphose spontaneously, but has lost the ability of responding with metamorphosis upon injections of thyroid-hormone, the species Triturus torosus employed in our experiment is one which undergoes normal metamorphosis.

Only one animal (a 3 of the series CCCXXXIII) proved suitable for the study of the Golgi apparatus in a truly dormant thyroid gland. This animal represents the control of the experimental animal CCCXXXIII b 12, to be described later on. The newts of this series had been received from Oregon, in August 1931, at a season at which the thyroids of the newts are completely dormant; before being experimented on they were kept in the laboratory for a period of nearly three months, a procedure which in our experience ensures a thoroughly inactive gland.

The thyroid cells of this control newt were low cuboid or flat, and did not contain either colloid droplets or Andersson vacuoles. The intrafollicular colloid showed only few, small vacuoles. The Golgi apparatus occupied an extreme apical position (fig. 1, Pl. 36). It consisted of an oval mass of a substance staining much darker than the rest of the cytoplasm. In this mass (or possibly on it—a question which I will not attempt to discuss here) were found deeply blackened bodies, of the shape of loops, filaments, or large spherical or irregular granules. This composition of the apparatus which will be seen in many other illustrations of cells in practically any one of the secretory phases, reminds one greatly of the differentiation of the apparatus into true Golgi substance and idiosomatic substance described in the male germ-cells of invertebrates by Bowen (1922) and by Beams and King (1932) in the nerve-cells of Orthoptera. Jacobs (1927) states that this structure was found so far only once, by Morelle, in vertebrates, and seems inclined to doubt its existence in vertebrates.

In the resting thyroid cell the apical Golgi network does not always represent the only element blackened by osmic acid. As is shown in fig. 1, Pl. 36, the cells contain, in addition to the network, a varying number of greyish granules which at present I consider of mixed nature representing mitochondria and ordinary cell granules, furthermore a few deeply blackened granules, often of irregular shape and of larger size than the greyish granules. If these latter granules are a part of the Golgi apparatus it would appear that even in the resting thyroid cell the Golgi substance is not strictly condensed into one compact network.

THE GOLGI APPARATUS IN THE NORMAL ACTIVE THYROID GLAND.

In a second series (CCCXXXIV) the conditions are of greater complexity. For years we have observed in our laboratory that adult newts received from Oregon during the winter months may show highly active thyroids soon after arrival in the laboratory. The newts used in series CCCXXXIV arrived in the laboratory January 4, 1932, and exhibited signs of high sexual activity. On the following day several pairs were found in copula. This state of sexual activity, however, passed off in a week and the animals exhibited a normal behaviour.

The animals of the series CCCXXXIV (a 1 to 4 and a 6 to 9) to be discussed in this chapter represent the controls of the animals CCCXXXIV b 2, 4, 6, and 7, which were injected with thyroid activator and will be discussed later on. The controls a 1–4 were not injected at all; the controls a 6–9 received injections of muscle extract prepared exactly like the anterior-lobe extract. The muscle extract had no effect upon the thyroid gland, as we have seen in previous articles.

Thyroids of a copulating male and female (a 1 and a 2), killed in the act of mating one day after arrival, were found, contrary to expectations, in an entirely inactive state, representing the extreme of a storage type. The follicles were of very considerable size and contained colloid which proved unusually hard, when the specimens were sectioned for histological purposes; the cells were greatly flattened out. Only Zenker and Champy preparations were made. In both cases Andersson vacuoles were absent, but in the thyroid of the female large numbers of intracellular colloid droplets were found.

Two other animals (a 3 and a 4) were killed, one on the second, the other on the third day after arrival. Their thyroids showed a markedly increased activity. Especially conspicuous were the large numbers of vacuoles in the intrafollicular colloid. Again only Chmapy and Zenker preparations were made. In the male the cells contained now some few Andersson vacuoles, but no colloid droplets. In the female the number of Andersson vacuoles was greater, and a moderate number of intracellular colloid droplets was present.

On the eighth day after arrival again two animals (a 6 and a 7), both males, were killed; these had received four intraperitoneal injections of beef-muscle extract. The thyroids now showed signs of considerable activity. The thyroids of one animal, of which Zenker and Champy preparations were made, possessed a highly vacuolized intrafollicular colloid and cuboidal cells containing no colloid droplets, but significant numbers of Andersson vacuoles. Of the other animal (CCCXXXIV a 7) a Zenker and a Nassonov preparation were made; collapsed follicles were found, the intrafollicular colloid was highly vacuolized, the cells were considerably distended and nearly 100 per cent. contained large numbers of Andersson vacuoles, but practically no colloid droplets. In many places signs of extrusion of liquid through the basal pole were visible. Apparently this gland was in a state of releasing the colloid from the follicles and excreting it through the basal ends of the cells. Nevertheless some cells were found which contained neither intracellular colloid droplets nor Andersson vacuoles, but were merely increased in height, possibly in preparation to become functionally active. Two such cells are illustrated in fig. 2, Pl. 36; as compared to the cells of the completely dormant gland (fig. 1, Pl. 36) the Golgi apparatus is very large, yet it occupies, in its entirety, an apical position. Again it consists of a deeply blackened true Golgi substance and of a dark-staining substance resembling Bowen's idiosoma of the invertebrate cell. Another still inactive cell has been illustrated in fig. 3, Pl. 36, because it shows the peculiarity which Nassonov has described (1923) as characteristic of the amphibian Golgi apparatus generally, and which he interprets as a cross-section through an epinuclear ring (corona).

In the great majority of the cells of this gland, vacuolized or containing compact cytoplasm, the substance impregnated by the osmic acid was distributed in the form of isolated fragments instead of presenting the shape of a network. Especially in the cells which contained no vacuoles or only a few, the isolated bodies were of spherical or irregular shape frequently larger than mitochondria. In the cells in which the cytoplasm had been replaced largely by vacuoles the black bodies were of filamentous shape (fig. 4, Pl. 36). The question may be asked whether these bodies are mitochondria or really the part of a Golgi apparatus.

Owen and Bensley (1929) pointed out that the osmic acid is not a specific stain for the Golgi apparatus, but may stain, in addition to other structures, the mitochondria, and does so quite often in cells in which it fails to impregnate the Golgi apparatus. That there might be a possibility of confusing the mitochondria with what is believed to be a fragmented Golgi apparatus has been alluded to repeatedly (Da Fano, 1922, p. 474; Jacobs, 1927, p. 367) although the difference in size is mostly relied upon as a means of differentiation. Nassonov (1923 and 1924) suggested the use of the Altmann-Kull technique which permits staining the mitochondria in osmic-acid preparations in which the Golgi apparatus has been blackened.

In order to make certain which of the osmiophilic bodies are Golgi substance and which mitochondria, we prepared for each of the glands impregnated with osmic acid after Nassonov a few slides stained with a mitochondrial stain. In our hands the Altmann-Kull technique did not prove very successful, whereas the Altmann-Bensley method (application of methyl-green after staining with Altmann's acid Fuchsin) gave very splendid results.

When treated with this technique, small bodies stained which were entirely different from the osmiophilic bodies. The fuchsinophilic elements were predominantly spherical, seldom a few rod-like mitochondria were mixed in with the spherical ones, no filaments stained with the fuchsin. The distribution of the mitochondria is entirely different from that of the osmiophilic pieces. The mitochondria are always distributed evenly; throughout the entire cytoplasm they are placed in regular intervals from each other. In the partitions between the lacunae they form rows in which the granules are placed in surprisingly uniform distances. The Golgi bodies, on the contrary, show a very irregular distribution. Moreover, the mitochondria are always strictly isolated from each other, whereas in the case of the filamentous Golgi bodies it can be observed, by focusing up and down, that at least many of the filaments are in reality only parts of a complex network, being continuous at some level of the preparation with adjacent filaments. Furthermore, a careful examination of the cells containing osmiophilic pieces of spherical or irregular shape revealed the presence, in many cells, of a matrix-like structure; a delicate network staining slightly darker than the cytoplasm and of great complexity was spread through the entire cytoplasm; in it are embedded the granules the distribution of which reflects merely the course of the fine trabeculae of the network. It is evident that the osmiophilic bodies of this gland are not mitochondria, but real Golgi substance.

Even so this substance occupied either a strictly apical position or, if scattered throughout the cell, still appeared to have a preference for the apical rather than for the basal pole.

One month and a half after arrival in the laboratory again one female (a 9) and one male (a 8) were killed, the former after nineteen, and the latter after seventeen muscle-extract injections. In the female (a 9) one gland was fixed in Zenker, the other in Champy. The colloid vacuoles were small and scarce; in both glands the cells were low cuboidal and contained neither colloid droplets nor Andersson vacuoles. The thyroids of the male (a 8) were fixed in Zenker and after Nassonov; in general structure they resembled the glands of the female. In both of these animals the thyroids were inactive, but the cells had not yet returned to the flat shape found in thyroids which have been storing colloid for a prolonged period of time. The Golgi apparatus in the male gland is illustrated in figs. 5 and 6, Pl. 36. In some cells (fig. 5, Pl. 36) the Golgi substance has the appearance of a network occupying an apical position; frequently it consists of the true blackened Golgi substance and, in addition, of the dark-staining core resembling the idiosomatic substance. In most of the cells, however, the cytoplasm contains, instead of a network, large numbers of individual bodies. This condition is illustrated in fig. 6, Pl. 36; in the larger cell the apical region was occupied by a dense mass of blackened granules, in which the individual bodies could not be well distinguished; it has been omitted in the illustration. In the smaller cell all the osmiophilic bodies visible at that focus are illustrated. In shape the granules are large irregular lumps or filaments (few) or smaller spherical granules. Of this thyroid Altmann-Kull as well as Altmann-Bensley preparations were made; in both cases the cytoplasm was completely filled with densely placed, but evenly distributed spherical granules. In size these were equal to the smallest spherical granules blackened with osmic acid, a fact quite distinct in such preparations which had not been completely bleached before being stained with the Altmann technique. Apparently only the large, irregular lumps visible in fig. 6, Pl. 36, are Golgi material. But, even when the blackened material as a whole is considered, it is evident that, while it is scattered throughout the cytoplasm, it is more densely placed at the apical pole; in fact in the small cell figured the basal region is free of osmiophilic material.

In the foregoing lot of newts a spontaneous activation of the thyroid took place after the animals had been in the laboratory for a few days, which attained a maximum in the newts examined about one week after arrival. At that time the thyroid cells were greatly vacuolized and secretion was extruded through the basal poles as evidenced in histological sections. One month later the thyroids had returned again to a resting stage, although the cells were still found cuboidal instead of flat as in the dormant gland.

The Golgi apparatus in the thyroids which excreted through the basal poles of the cells was enlarged as compared to the Golgi apparatus in the truly inactive thyroid (CCCXXXIII a 3); in many of the highly active cells it was broken up into fragments. After return to a resting stage the Golgi apparatus presented much the same appearance as in the active glands.

Although one of the two thyroids impregnated with osmic acid excreted through the basal cell-poles, the Golgi apparatus occupied either a strictly apical position or, when fragmented, did not show a special preference for the basal pole.

THE GOLGI APPARATUS OF THE THYROID STIMULATED BY THE THYROID ACTIVATOR.

In the chapter on the Golgi apparatus of the normal resting thyroid cell the Golgi apparatus of one normal animal (CCCXXXIII a 2) was described and illustrated. The experimental animal of the same series, CCCXXXIII b 12, received four injections of thyroid activator and was killed one day after the last injection. One thyroid was fixed in Zenker, the other one was impregnated after Nassonov. Many follicles were collapsed, the intrafollicular colloid greatly vacuolized, the cells were greatly enlarged, high cuboidal or columnar, and vacuolized to an extreme degree. The vacuoles are not of a regular, spherical shape, but present themselves as large, irregular lacunae of fluid; the cytoplasm is reduced to delicate partitions between the lacunae. In addition many basal vacuoles were protruding from the cells into the blood spaces, indicating an active extrusion of liquid from the basal ends of the cells. Briefly this thyroid was in a state of activity, which might be considered representative of the reversal of the secretory polarity from the apical to the basal pole. All cells contained bodies well impregnated with osmic acid. In fig. 7, Pl. 36, two cells are illustrated in which the liquid material has accumulated only on one side of the nucleus; it forms large lacunae extending from the apex to the base. In both cells the Golgi apparatus is represented largely by a network much enlarged in volume as compared to the normal control animal (fig. 1, Pl. 36). As in the normal resting gland it shows distinctly the 'idiosomatic' substance. In one cell it occupies a strictly apical position, in the other cell the major portion lies at the apical side of the nucleus, but a few smaller fragments are lodged in one of the cytoplasmic strands, close to the base of the cell, but not basal to the nucleus. In fig. 8, Pl. 36, a cell of the same thyroid is illustrated; it represents the most frequent cell-type of this gland and is conspicuous by the extreme vacuolization of the cytoplasm, resulting in the formation of lacunae. The distribution of the osmiophilic substance is quite characteristic; the osmiophilic fragments are scattered throughout the cell and always are lodged in the delicate cytoplasmic partitions separating the lacunae of liquid from each other. In studying the lacunation of the thyroid cell, which takes place after injection of thyroid activator, one cannot fail to recognize the close resemblance between it and the vacuolization of other gland-cells. In regard to the relation between the lacunation process and the distribution pattern of the Golgi apparatus the resemblance between the lacunation of the thyroid cell and that of the mucous cells of the salivary glands of Limax, so well described by Bowen (1926*a*), is particularly conspicuous. Bowen mentions that it is quite difficult to decide whether the Golgi network merely expands to such an extent that only sections of its trabeculae are visible in one preparation, or whether the individual bodies represent really isolated fragments. In the case of the thyroid cells represented in fig. 8, Pl. 36, focusing up and down does not permit establishing a continuity between the individual pieces; I feel convinced that at that stage a real fragmentation has taken place. A similar distribution pattern was observed by Weatherford (1929) in the lactating mammary gland. Weatherford, adopting the viewpoint of Nassonov, considers this distribution, owing to the apparent close relationship between vacuoles and Golgi substance, as indicating that the latter is somehow involved in the manufacture of the vacuoles. While there may exist a functional relationship between the Golgi apparatus and secretion product, the mere topographical proximity of the two may not be sufficient evidence to prove a functional relation. The fact must not be overlooked that the cytoplasm and all its other constituents (especially the mitochondria) show identically the same distribution. Expressed in terms of a simple unbiased observation this relation may be summarized in the following sentence: the distribution of the Golgi substance repeats accurately the distribution of the cytoplasm. In this connexion it is of interest that Beams and King, in a recent study of the Golgi apparatus of the ganglion cells of insects (1932), are somewhat sceptical regarding the specific secretory function of the Golgi apparatus. According to these investigators the cytoplasm as a whole secretes; the Golgi apparatus partakes in this function only as one of the component parts of the cytoplasm.

While the thyroid of this animal represents a stage of highest activity accompanied by excretion through the basal pole, in not one cell could a true reversal of the Golgi apparatus be found. The Golgi apparatus is either net-like and strictly apical, or fragmented and evenly scattered in the cell, following the ramifications of the cytoplasm.

Newts of the same lot from which the control animals 'CCCXXXIV a' were taken were injected with thyroid activator. The first injection was made two days after the animals were received in the laboratory, in the case of b 2, and on the same day at which the animals were received in the case of b 4, b 6, and b 7. As described in the previous chapter, the control newts showed a spontaneous activation of the thyroid gland which attained a maximum about eight days after arrival in the laboratory. In the experimental animals, therefore, the effects of the thyroid activator upon the thyroid were added to the effects of the spontaneous activation. As will be discussed in another article, this procedure does not necessarily result in a quantitative summation of the activating effects which are observed in the dormant thyroids after injections of thyroid activator.

Of four of the animals (b 2, 4, 6, and 7) injected with thyroid activator the right thyroid was fixed and impregnated after Nassonov, while the left gland was fixed in Zenker.

CCCXXXIV b 2 received two injections of thyroid activator and was killed just two days before the control CCCXXXIV a 7 (figs. 2, 3, and 4, Pl. 36) was examined, an animal in which the spontaneous activation showed a maximum degree. In the experimental animal, however, the thyroids were found less active than in the controls killed two days later. The reasons for this fact will be discussed more conveniently in another article; but it may be stated briefly here that this condition is due to the overlapping of periodical cycles induced by repeated injection. All follicles were well distended, but the intrafollicular colloid was considerably vacuolized. The cells were mostly low cuboid and contained only very few Andersson vacuoles and few intracellular colloid droplets. No structures were found giving reliable evidence of basal excretion. In the low cuboidal cells the Golgi apparatus presented much the appearance it had in the dormant gland (fig. 1, Pl. 36). In the higher cells it had the shape of a network (fig. 9, Pl. 36). Lying between such cells and alternating with them in no definite order were found other cells, usually higher, in which only osmiophilic fragments in large numbers were scattered throughout the cell-body, very much like the condition described for the glands a 7 and a 8 (fig. 6, Pl. 36). Many more small spherical or irregular granules were found, however, among the larger lumps; these smaller bodies had much the appearance of mitochondria, a question which was not examined in this gland. It was quite evident, however, that in not one cell did the blackened bodies show a preference for the basal pole; on the contrary there was always a greater massing of the bodies in the apical region.

An important peculiarity was observed in the Golgi apparatus of this gland as may be seen in figs. 9 and 10, Pl. 36. In those cells in which colloid droplets were present, the trabeculae of the Golgi net frequently surrounded the droplets completely, a condition which resembles much the pictures upon which Nassonov based his theory of the secretion activity of the Golgi apparatus (Nassonov, 1923, 1924).

A second experimental animal (CCCXXXIV b 4) was killed after four injections, eight days after its arrival in the laboratory, on the same day on which the control animal CCCXXXIV a 7 was examined, an animal in which the spontaneous activation of the thyroid gland had attained a maximum degree (figs. 2, 3, 4, Pl. 36). The thyroids of the experimental animal are of especial importance inasmuch as there are present at least two different types of cells which can be correlated with definite functional phases; they are associated with different types of follicles. The gland consists of considerably large, sometimes completely distended, sometimes slightly folded follicles which contain great quantities of colloid and numerous large colloid vacuoles; and of completely collapsed follicles which contain either no colloid at all or only colloid vacuoles. The walls of the large follicles are made up of high columnar cells vacuolized in different degrees, but predominantly at a maximum degree. The walls of the collapsed follicles consist of high cuboidal cells; the cytoplasm of these cells is compact, contains no vacuoles, but a great many colloid droplets. Evidently the vacuoles in the cells of the large follicles are the follicular content which has been resorbed by the cells, and is being liquefied in preparation for its extrusion through the basal pole into the blood sinusoids. Histological evidence of basal excretion is very ample in the large follicles; in numerous places large quantities of the liquid cell-content penetrate from the basal cell-ends between the connective-tissue fibres into the blood sinuses. In the collapsed follicles the process of colloid release has come to an end; the colloid masses which were the last to enter the cells through the apical poles had not been extruded through the basal pole, but remained at the apical pole and solidified here to form the colloid droplets. The cells are apparently in a state of temporary rest waiting for a new stimulus by the action of which the secretion current will be reversed and the empty follicles refilled.

Corresponding to this differentiation of the follicles and their cells is the differentiation of the Golgi apparatus into two main types. In the tall cells of the large follicles the Golgi apparatus appears in several variations all of which can be traced back to the network-type. In figs. 11, 12, 13, 14, and 15, Pl. 36, these variations have been illustrated in series. All of them were taken from the same follicle, yet the individual members of the series may be looked upon as different developmental stages. In the first stage (fig. 11, Pl. 36) the apparatus has the shape of a closed network greatly hypertrophied as compared with the resting condition (compare with fig. 1, Pl. 36). Its trabeculae surround completely some of the most apical vacuoles. In the next stage (fig. 12, Pl. 36) the network has become open towards the apical pole; the vacuoles are closely surrounded by the trabeculae except on the apical side, where they are free and closely approximated to the apical cell-wall. In the third stage (fig. 13, Pl. 36) the network shows still further hypertrophy. The cell is much increased in size owing to the increase in fluid which has now fused to form large lacunae. The Golgi net, which has opened up still further towards the cell apex, surrounds the lacunae closely leaving them free only at the apical side; here only a thin partition separates the fluid from the follicular lumen. It is noteworthy that a small vacuole has formed at the basal side of the apparatus; this vacuole is free at the basal side, but otherwise lodged deeply in the network. In the next stage (fig. 14, Pl. 36) the cell-plasma is still more extensively vacuolated; the vacuoles are now extending far towards the base of the cell, a change foreshadowed by the vacuole in fig. 13, Pl. 36, which is free at the basal side. A small epinuclear portion of cytoplasm is left unvacuolated, exactly as described by Bowen (1926*a*, p. 94) for the mucous cells of the salivary gland of Limax. In it is lodged the major portion of the Golgi apparatus; it no longer has the shape of a network, but is broken up into large numbers of individual bodies. These are strictly separated from each other and are spread out along the thin cytoplasmic partitions left between the lacunae. In the next stage (fig. 15, Pl. 36) the lacunation of the cell has attained a maximum degree and the cell is much distended. Only very fine cytoplasmic partitions are left between the lacunae which occupy every available space (the nucleus of this cell was not contained in this section). The Golgi substance is broken up into bits which, owing to the growth of the lacunae, are now widely separated from each other.

The reader will find it interesting to compare the changes of the Golgi apparatus as related to the changes of the cell of the thyroid gland with the descriptions given by Bowen (1926*a*) for various gland-cells which are known to excrete only through the apical pole. The resemblance between the thyroid cell and these gland-cells is surprisingly great. While it is not intended to enter here upon an analysis of the secretion processes of the thyroid gland, it may be reiterated that the thyroid cells described here present histological peculiarities which seem to be the structural expression of basal and of apical excretion. Nevertheless the Golgi apparatus, as may be seen from an inspection of the illustrations, is located apically either in its entirety or with its main portion; a reversal of its position has not been observed in a single cell.

In the cuboidal cells lining the empty collapsed follicles the Golgi apparatus is never found to have the shape of a network, but is completely fragmented into large numbers of black bodies. As there are no vacuoles in these cells the fragments lie close together. These fragments are most often solid, black bodies much like those illustrated in fig. 6, Pl. 36, for another thyroid; but in a small number of cells they have the shape of the so-called ring-like bodies consisting of a deeply blackened ring and of a dark-staining core (compare fig. 19, Pl. 36).

Regarding the fact that the cuboidal cells of the empty follicles represent the approach of the end of the secretory cycle, it is of interest that a similar fragmented condition has been reported by other investigators at the end of the secretory cycle in other gland-cells (Bowen, Weatherford, and others). The ring-like fragments resemble closely those illustrated by Weatherford in his figs. 13, 16, 32, 33, and 34. In the first two figures which represent a stage just before the maximum activity is attained, Weatherford interprets the black rings as 'meshes' of the Golgi apparatus and the dark substance contained in them as vacuoles at the inception of their development. In the cells of the thyroid the contents of the black rings has no similarity with vacuoles; it resembles much the dark substance termed idiosomatic substance by Bowen and visible in the thyroid cells also when the Golgi apparatus has the shape of a network.

The next animal (CCCXXXIV b 6) was killed after six injections of thyroid activator, four days after the control thyroids (a 7) had attained a maximum degree of activity. The thyroids resemble much those of the previous animal. Of importance is the fact that in no case a reversal of the position of the Golgi apparatus was found, although ample histological evidence of basal excretion activity is furnished by inspection of the slides.

The last animal (CCCXXXIV b 7) was killed after fourteen injections of thyroid activator, one month after arrival in the laboratory and eight days before the inactive glands of the control 'a 8' were examined. Again this animal is of considerable interest, as the thyroid cells show structures which are suggestive in regard to the direction of the secretion current. In the last two animals, especially b 4, there existed a distinct differentiation of the cells in regard to large distended and small empty follicles. In the former the cells were lacunated and the colloid was released from the follicles by basal excretion. In the latter the cells were filled with mostly apical colloid droplets suggesting a cessation of excretion. In the present thyroid there are again two kinds of follicles present, larger, considerably folded follicles containing a good deal of colloid; and collapsed follicles which, however, contain mostly some colloid. Among the cells too there exists a distinct differentiation into two types, but no correlation between them and the type of follicle can be made out. On the contrary the two types of cells are represented in equal numbers in all the follicles.

In one cell-type (fig. 18, Pl. 36) the cytoplasm contains vacuoles. These vacuoles are, however, different from the vacuoles and lacunae of the vacuolized cells present in the previous thyroids. Always the vacuoles are confined to the apical region. They are relatively small and of extremely regular spherical shape. The cytoplasm intervening between them is ample and does not present the appearance of partitions between the vacuoles (figs. 18 and 21, Pl. 36). It is probable that the origin of these vacuoles is different from that of the larger lacunae observed in the cells of the previous thyroids. This opinion is suggested not only by the appearance of the vacuoles, but also by an inspection of the second type of cells.

In the second cell-type the most conspicuous feature is the presence of intracellular colloid droplets, often in large numbers. These are rarely (fig. 22, Pl. 36) of the simple nature of the droplets found in the cuboidal cells of the previous thyroid. In most cells they present a very characteristic condition (fig. 16, Pl. 36). Droplets are found in the peripheral zone of which is fitted in a small vacuole. In other instances the vacuole is much larger and is associated with a crescentic droplet fitted closely on to the periphery of the vacuole. Some vacuoles may be associated with two crescentic droplets. Or the vacuole may be surrounded entirely by the substance of the droplet (fig. 16, Pl. 36). The droplet may form a mere narrow crescentic cap on one side of the vacuole much like the cap of Golgi substance associated with droplets in the cells of the avian oil-gland (Bowen, 1926*b*, fig. 55), but different from Golgi substance inasmuch as these caps will stain with Heidenhain-Mallory after Zenker fixation and with fuchsin in the Nassonov preparations. Finally the droplet together with its vacuole becomes enclosed in a large vacuole and disintegrates into a faintly-staining granular mass (figs. 17 and 20, Pl. 36). Especially in Zenker preparations stained with Heidenhain-Mallory fine transitions in staining capacity from a deep blue to the faintest blue shade can be found among these droplets. The final result of this liquefaction would be the vacuolated cell-type illustrated in fig. 18, Pl. 36. It is evident that in this thyroid the droplets deposited within the cell after release of the colloid from the follicles are dissolved in preparation of a refilling of the follicles by way of an apical excretion.

In the larger number of cells only Golgi pieces are present. These may be solidly black (figs. 20 and 21, Pl. 36). But in most cells and especially so in the cells in which the liquefaction of the droplets is still in progress, the Golgi bodies are ring-like (fig. 19, Pl. 36). A definite and constant relation between droplets and vacuoles on the one hand and the Golgi bodies on the other hand is not apparent.

In a small number of cells, however, conditions are entirely different. Here the Golgi apparatus has the shape of a large hypertrophic network (figs. 22 and 23, Pl. 36); and this may be closely associated with the droplets (fig. 22, Pl. 36) or vacuoles (fig. 23, Pl. 36). In the latter case the vacuoles are of the type of the lacunae described in the previous thyroid.

Attention should be called to the presence in this thyroid of numerous small granules impregnated with osmic acid (figs. 21, 22, 23, Pl. 36). Several preparations were bleached and stained after Bensley. The mitochondria which stained in these preparations (figs. 17, 18, Pl. 36) had no similarity with the spherical granules shown in figs. 22 and 23, Pl. 36. Yet it is certain that the blackened granules are not Golgi substance.

While an analysis of the possible functional relation of the Golgi apparatus in this gland is not entirely successful, largely on account of the presence of the two cell-types illustrated in figs. 22 and 23, Pl. 36, it is quite evident that, although the Golgi apparatus may be scattered throughout the cytoplasm, its position is never reversed from the apical to the basal pole.

DISCUSSION.

The foregoing study of the Golgi apparatus in the cells of the amphibian thyroid does not pretend to offer any new aspects of the structure and significance of the Golgi substance. It does, however, contribute one important fact: the Golgi apparatus of the activated thyroid gland occupies either a strictly or predominantly apical position; in not one of the secretion phases of the thyroid cell does it show a reversal of its position. The non-reversibility of the Golgi apparatus has been demonstrated previously by other investigators; but it gains an added significance by the work presented here, as the Golgi apparatus was examined in all the secretion phases of the thyroid cell, and as it was possible to show that, at a certain stage of the secretory cycle, the cells present histological structures which, with a high degree of probability, are the structural equivalent of a basal excretory activity. While the Golgi apparatus may be concerned with the manufacture of the secretion products, as Nassonov, Bowen, Bensley, Weatherford, and others believe, its position certainly does not permit us to know at what pole the secretion product is excreted.

In the thyroid gland of the newt the Golgi apparatus presents all the essential characters of shape, structure, and distribution which have been described in other gland-cells. One feature appears particularly noteworthy regarding the structure of the apparatus. The composition of the Golgi apparatus in the invertebrate cells is believed to be of a duplex nature; the true Golgi substance staining black after osmium-impregnation is one component; the other component is believed to be a substance staining much darker than the cytoplasm, but not blackening. The latter substance has been termed idiosoma by Bowen. In vertebrates the idiosomatic substance has been observed only rarely. There is no doubt that the dark-staining substance found in the Golgi apparatus of the newt resembles greatly the idiosomatic substance. In the present study no experimental evidence was obtained to indicate whether or not the two components of the Golgi apparatus are actually two different substances. In the light of the work of Owen and Bensley (1929) showing that the blackening after a stay in osmic acid is due to an adsorption of precipitated particles one would often feel inclined to consider the idiosomatic component as the true Golgi material on the surface of which the black particles have been adsorbed; this explanation applies particularly to the ring-shaped particles which are nothing but an optical section through a particle, the outer surface of which is covered by a thin black shell.

Considering the differentiation of the apparatus in relation to the secretory phases of the cell it was possible to ascertain that in the resting cell the apparatus is comparatively small and compressed in an apico-basal direction. In cells preparatory to basal excretion the Golgi net enlarges greatly in an apico-basal direction and its trabeculae become very stout. In cells in which fluid has accumulated in lacunae, and which excrete through the basal pole, the apparatus breaks up into fragments which frequently have the shape of long, thin threads or filaments. In cells which have ceased to excrete basally, and in which the remaining fluid has become condensed into intracellular colloid droplets, the fragments of Golgi substance become short and lumpy. When the droplets are redissolved in preparation for apical excretion to refill the follicles, the fragments are predominantly of ring shape. A complete picture of the changes of the apparatus during the secretory cycle cannot be given, however, until preparations are available in which the overlapping of the effects of repeated injections of thyroid activator has been avoided.

The mechanism of the secretion processes of the thyroid cell is still a matter of debate. Of particular interest is the question whether the reabsorption and passage of the colloid through the cell, and the transformations of the secretions within the cells, the liquefaction and solidification of the materials, are caused by the presence or absence, respectively, of currents of liquid, by the simple forces of diffusion and osmotic pressure, or whether they are performed secondarily by active metabolic processes of the cells. The latter alternative would appear very probable if it could be demonstrated that the Golgi apparatus is directly concerned in the transformation of the secretion products. While a definite answer to these questions is deferred for a later publication, when more ample material will be available, it might be stated here that a topographical relationship as described by Nassonov has been found in several instances (figs. 12–15 and 22 and 23, Pl. 36). In most cases, however, the relation between the Golgi apparatus and the secretion products did not seem by any means specific, but was shared in by the mitochondria and the cytoplasm as a whole. The Golgi apparatus most frequently shows a distribution which merely repeats more or less accurately the distribution of the cytoplasm in its entirety.

SUMMARY.

1. The thyroids of the adult Californian newt, Triturus torosus, were examined in Zenker, Champy, and Nassonov preparations, in one series in which these glands were entirely at rest, in another series in which they underwent a spontaneous activation and in a third group in which activation had been forced by intraperitoneal injections of thyroid activator.

2. As in invertebrates so in the newt the Golgi apparatus appears to consist of two components, of one which is deeply blackened, and of another one which stains much darker than the cytoplasm and corresponds to Bowen's idiosomatic substance. The former frequently forms a shell around the latter as a core. The problem has been discussed in the light of the work published recently by Owen and Bensley.

3. Only incomplete observations are available concerning a possible relation between Golgi apparatus and functional phase of the cell.

(*a*) In the resting condition the Golgi apparatus is relatively small and compressed in an apico-basal direction.

(*b*) In preparation for colloid release through the basal cell-ends, the Golgi apparatus enlarges greatly in an apico-basal direction and its trabeculae become stout.

(*c*) In the cells in which fluid has accumulated in large lacunae and is excreted through the basal cell-ends, the Golgi apparatus begins to become fragmented into long, slender pieces.

(*d*) In the cells in which basal excretion has ceased and the remaining liquid has been condensed into stainable droplets, the fragments are transformed into short, thick, and lumpy pieces.

(*e*) When the colloid droplets are redissolved and transformed into vacuoles for the purpose of refilling the follicles, the Golgi bodies appear as black rings around a dark core.

4. In most instances the topographical relation existing between the Golgi apparatus and the secretion products is not specific. The distribution of the Golgi material represents merely an accurate repetition of the distribution of the cytoplasm.

5. In some instances, however, a close relationship is found between the Golgi apparatus and the secretion products (figs. 12–15, 22, 23, Pl. 36).

6. In no case does the Golgi apparatus show a reversal of its position from the apex to the base of the cell. In cells which are in an active state of basal excretion the Golgi apparatus may be strictly apical. Its position does not convey a knowledge of the excretion polarity of the cell.

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EXPLANATION OF PLATE 36.

All figures illustrate thyroid cells of the adult Californian newt, Triturus torosus.

All illustrations are made from material fixed and impregnated by osmic acid after the technique recommended by Nassonov. In every case the tissue had been left in 0·2 per cent. osmic acid for forty-eight hours at approximately 35° C.

All illustrations are carbon pencil drawings, made from the original preparations by the aid of a camera lucida. The nuclei are frequently diagrammatic. The Golgi apparatus was accurately outlined, in every case, in relation to size, shape, and distribution. Of the mitochondria and other granules only a few were outlined to obtain a correct idea of their size, shape, and general pattern of distribution; the remaining number were put in free hand.

Figs. 1–11 and 20–23, Pl. 36, were drawn under a Zeiss ocular ×20 and Zeiss oil-immersion apochromate ×90, 1·4 aperture, figs. 12–19, Pl. 36, under a Zeiss ocular ×20 and Spencer oil-immersion 1·8 mm., giving magnifications of 1,800 and 2,200 respectively. Since the drawing board was kept below the level of the stage, the magnifications were still further increased, to 2,450 and 3,000 respectively. In reproduction the originals have been reduced.

In all of the illustrations the apical pole (lumen end) of the cell faces upward, the basal pole downward, except Fig. 19 in which the apical pole is to the left.

Fig. 1 (CCCXXXIII a 3).—Cells from a completely dormant thyroid gland. The black granules are probably parts of the Golgi apparatus; the grey granules represent probably both mitochondria and ordinary cell granules.

Fig. 2 (CCCXXXIV a 7, A).—From a thyroid activated spontaneously to a high degree of activity. Two cells showing an increased height in preparation to secretory activity. Golgi apparatus greatly enlarged.

Fig. 3 (CCCXXXIV a 7, B).—From same gland. Inactive cell showing corona-like Golgi apparatus.

Fig. 4 (CCCXXXIV a 7, C).—From same gland. Highly vacuolized cells (vacuoles not illustrated), containing a filamentous Golgi apparatus.

Fig. 5 (CCCXXXIV a 8, B).—From a thyroid returning to a dormant state after having gone through a period of spontaneous activity. Low cuboid cell showing compact cytoplasma and epinuclear network of Golgi.

Fig. 6 (CCCXXXIV a 8, C).—From same thyroid. Cells containing neither vacuoles nor colloid droplets. Golgi apparatus fragmented. Only the larger irregular lumps are Golgi bodies, the small spherical granules are probably mitochondria. In the larger cell a dense apical accumulation of blackened granules was omitted in the drawing.

Fig. 7 (CCCXXXIII b 12, 5 a).—From a thyroid stimulated by four injections of thyroid activator to maximum degree of activity. Two cells containing large paranuclear lacunae. Golgi apparatus only slightly fragmented, and placed at apical pole.

Fig. 8 (CCCXXXIII b 12, 4 c).—Same thyroid. Completely lacunated cell. Golgi apparatus fragmented, imitating in its distribution pattern that of the cytoplasm. No reversal of position of Golgi apparatus.

Fig. 9 (CCCXXXIV b 2, A).—From a thyroid stimulated by two injections of thyroid activator. A cell containing a net-like Golgi apparatus and intracellular colloid droplets. Note the conspicuous relationship between these two elements.

Fig. 10 (CCCXXXIV b 2, B).—From the same thyroid and showing the same condition as fig. 9.

Figs. 11–15 (CCCXXXIV b 4, 1, 2, 3, 4, and 5.)—From a thyroid stimulated by four injections of thyroid activator to an extreme degree of functional activity.

Fig. 11.—The Golgi apparatus has the shape of a closed net, surrounding completely, with its meshes, the vacuoles.

Fig. 12.—The Golgi apparatus is open towards the lumen side; the vacuoles are free at the lumen side.

Fig. 13.—The Golgi apparatus has greatly increased in volume; it is open at the lumen side.

Fig. 14.—The vacuoles have expanded throughout the cell, also towards the base. The Golgi apparatus is fragmented; its major portion lies in the small epinuclear remnant of cytoplasm.

Fig. 15.—The nucleus is not visible in this section. The vacuoles have partly fused to form large lacunae, a condition representing the maximum degree of cytoplasmic vacuolization. The Golgi apparatus has diminished in volume; it is completely fragmented, but still its major portion lies apically.

Figs. 16–18 (CCCXXXIV b 7, A, B, and C), taken from a thyroid which had been stimulated by fourteen injections of thyroid activator. Three stages of the liquefaction of the intracellular colloid droplets are shown.

Fig. 16.—Several steps showing the change of the droplet into a vacuole.

Fig. 17.—The droplets are almost completely dissolved; only a granular mass of faintly-staining material is left within the vacuoles.

Fig. 18.—Finally a cell results which contains at the apical end numbers of regularly spherical vacuoles corresponding in size to the droplets from which they developed.

Figs. 19–23 (CCCXXXIV b 7, 6, 3, 2, 1, and 4).—Taken from the same thyroid as figs. 16–18.

Fig. 19.—Cuboidal cells containing colloid droplets in progress of liquefaction and ring-shaped Golgi bodies.

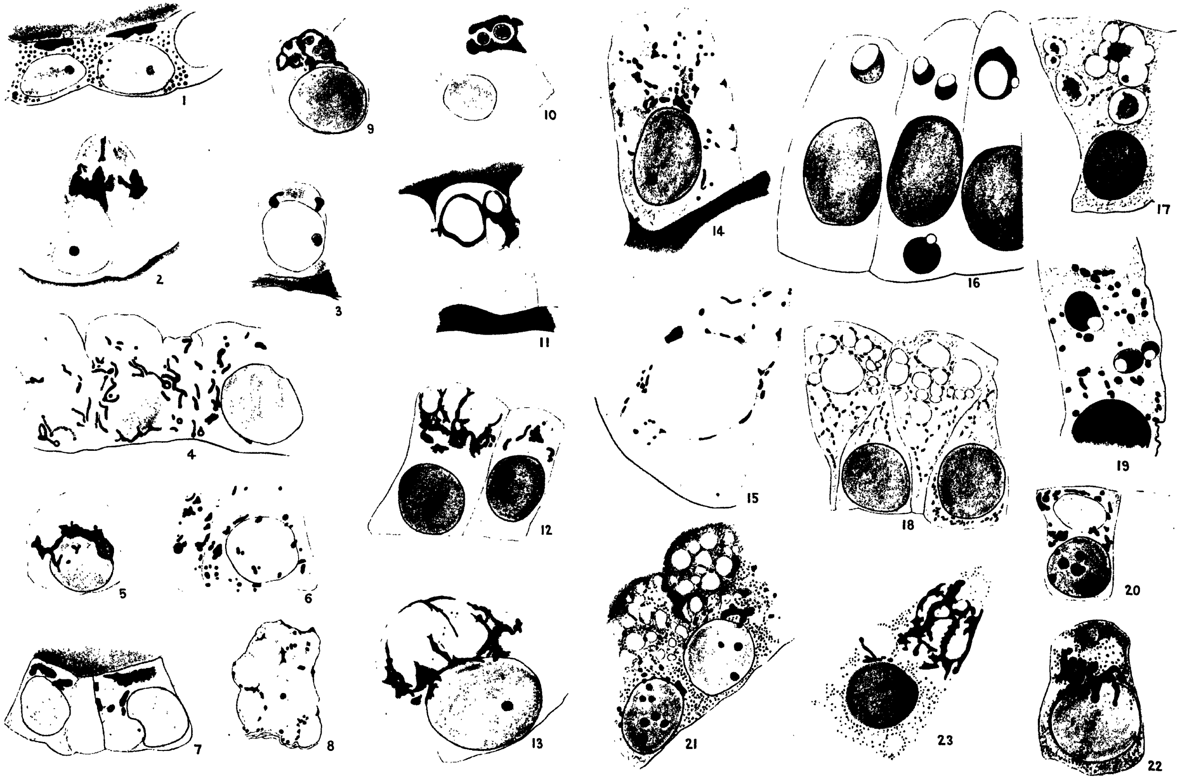
Fig. 20.—Cell containing a large, very faintly-staining apical vacuole which is filled with remnants of a colloid droplet. Fragments of Golgi apparatus are solid black.

Fig. 21.—Vacuolated cell. Golgi substance fragmented into black bodies.

Fig. 22.—Golgi apparatus net-like and closely associated with colloid droplets.

Fig. 23.—Hypertrophied Golgi net closely associated with vacuoles.

Note.—I am greatly indebted to Mr. Robert Fox of Greenwald, Inc., Baltimore, for permission to use the facilities of the abattoirs of his concern and for his generosity in placing at our disposal all the glands we needed.



E. Uhlenhuth, del.

The Formation of Melaniridosomes in healing wounds of Haemulidae.

By

George Milton Smith,

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With 4 Text-figures.

THE earlier studies of the pigment cells of fishes by v. Siebold (1863), Pouchet (1876), Leydig (1895), were followed by a long series of important investigations by Ballowitz (1893–1924) upon which a large part of our present knowledge of the complicated morphology of the teleost skin rests. More recently, information on this subject has been extended by the work of Kurz (1920), Becher (1924), Schnackenbeck (1925), Kann (1926), Jost (1926), Panu (1929), Graupner and Fischer (1933).

Ballowitz (1893) was the first to demonstrate in fishes the anatomical relationship of nerves to pigment cells. He recognized further that pigment cells (melanophores, erythrophores, xanthophores, iridocytes or guanophores) might exist in various combinations with one another to form definite cell-masses, designated by him as chromatic organs (Ballowitz, 1913). One of the simpler and commoner forms of chromatic organ is the melaniridosome, where a single melanophore lies in contact with one, several, or a mass of iridocytes. Thus the central part of a single melanophore may become surrounded to a great extent by these closely grouped iridocyte bodies, with long black pigmented processes projecting through and beyond the iridocyte mass (Text-fig. 1).

While studying inflammation and wound healing in two species of Bermuda grunts (Haemulidae) it was noted that, at certain stages of repair, isolated melanophores became associated with iridocytes to form melaniridosomes similar in appearance to those found normally in the corium of these fishes. The present

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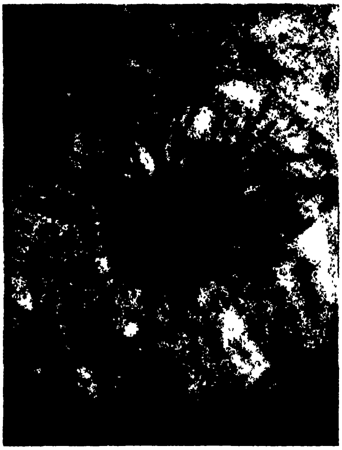
[1] Aided by a grant from the Blossom Fund, Yale University.

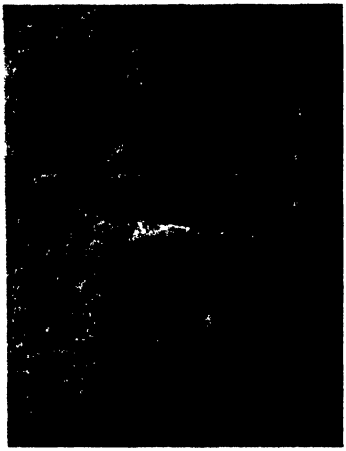
paper aims to describe briefly this association of melanophores and iridocytes to form melaniridosomes, as seen under experimental conditions.

METHOD.

All my observations on living fishes, comprising the largest share of the work, were made during the months of October 1932 and May 1933 at the Marine Biological Station, St. George's West, Bermuda, through the courtesy of Dr. John F. G. Wheeler, Director. The temperature of the tanks of sea-water was approximately 68° F. to 70° F. for both months.



TEXT-FIG. 1.



TEXT-FIG. 2.

1. Isolated melaniridosome of the corium of Bathystoma aurolineatum. The body of the melanophore lies buried in a spherical mass composed of small iridocyte bodies. The processes of the melanophore, in a state of expansion, project beyond the spherical mass of iridocytes into surrounding tissues. Microphotograph made from fresh tissue through surface epithelium. × 500.
2. Type of T-shaped wound employed in experiments, 12 hours after incision. No growth of new melanophores has occurred. Microphotograph × 8·5.

The two species of Haemulidae or grunts used in the experiments were Bathystoma aurolineatum (white grunt) and Haemulon sciurus (yellow grunt). According to Beebe and Tee-Van (1933) both represent common Bermuda species. They show melanophores in great abundance. For the most part these lie embedded, each in a conglomerate mass of iridocytes through which the black pigmented dendrites of the melanophores extend upward and outward with various degrees of expansion and contraction. Where massing of melanophores is dense, the iridocytes clustering about melanophores are loosely continuous with other iridocytes which extend as a thin iridescent layer slightly superficial to melanophores. In certain regions, however, like the head, conjunctiva, abdomen, and base of fins, the melaniridosome stands out as a typical isolated small chromatic organ exhibiting in direct light a brilliant metallic lustre.

Wound healing was studied in 150 fishes, from 3 to 6 inches in length. A small linear or T-shaped incision, measuring from ½ to 1 inch, was made on the left side of the body, near the dorsal fin. The horizontal limb of the T-shaped incision was above the lateral line, while the vertical limb divided this structure (Text-fig. 2). Daily examinations were made up to the end of three weeks. Numerous specimens were fixed in formalin 10 per cent. These were selected to represent wounds at time intervals of 4, 8, 12, 24 hours, and after that daily intervals to 10 days. Serial sections, cut in a longitudinal or transverse direction, were made of such specimens, and were stained with a large variety of stains. Information was best obtained, however, from direct microscopic inspection of the wound itself, or from fresh preparations of tissue made from the living fish by excising small groups of cells and suspending these in physiological saline or sea-water.

RESULTS.

The early changes in the wound, the appearance of inflammatory cells, the various steps in regeneration of tissues, followed rather closely the observations of Schmincke (1907). These changes are not described in detail in the present report, where attention is directed solely to newly formed melaniridosomes.

Degenerating melanophores at the margin of the wound were noted as early as four hours after incision was made. Small amounts of detritus of black pigment were found frequently in various parts of the wounds at practically all stages, even when healing was nearly completed.

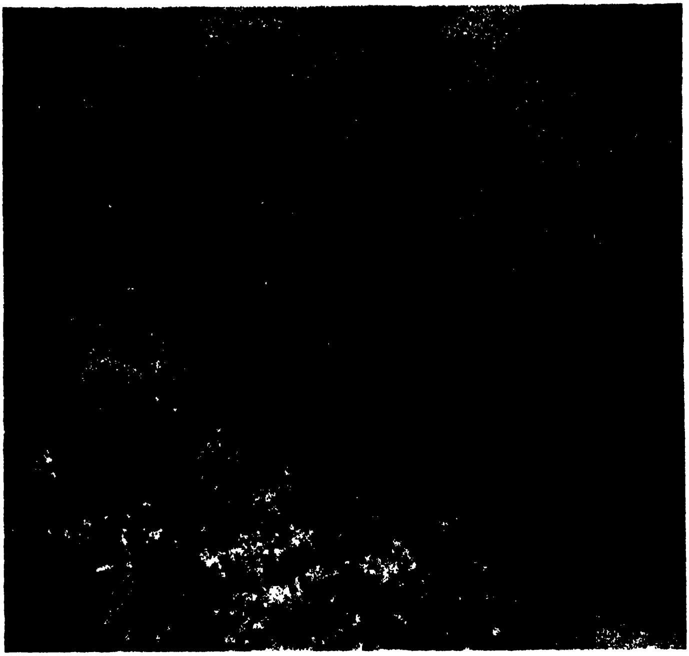
Newly formed melanophores were seen at the margin of wounds as early as the fifth day. They varied in numbers for different fishes. For the most part, they formed cells delicate in structure, lightly or deeply pigmented with melanin granules, their irregular processes extending out into the surrounding tissues. They were single or in small groups with interlacing dendrites.

At about this same time of wound healing, or even earlier (fourth day), small crystalline bodies resembling single or collections of iridocyte bodies were noted here and there in the wound itself. These were small, oval, or rod-like in shape and gave a white silvery iridescence in direct light. Later (6–10 days) there appeared often a dense massing of these crystalline bodies at the edges and in the substance of the wound, giving the impression that iridocytes were massed in large numbers for the process of repair. Soon after the appearance of melanophores and iridocytes (5–6 days) at the surface of the wound, a close approximation of these two types of tissue was noted. Melanophores were found with iridocytes clinging to the tips of dendrites, or with collections of iridocytes lying between the irregularly outstretched dendrites, with here and there an iridocyte located near the centre of the melanophore (Text-fig. 3).

In the late stages of healing (10–14 days) large masses of iridocytes were observed clustered about the centre of the melanophore, such combinations of melanophore with iridocytes resembling the melaniridosome seen in normal corium of these fishes (Text-fig. 4, Group B, figs. 8 and 9, and also Microphotograph 3, X, Y, Z).

COMMENT.

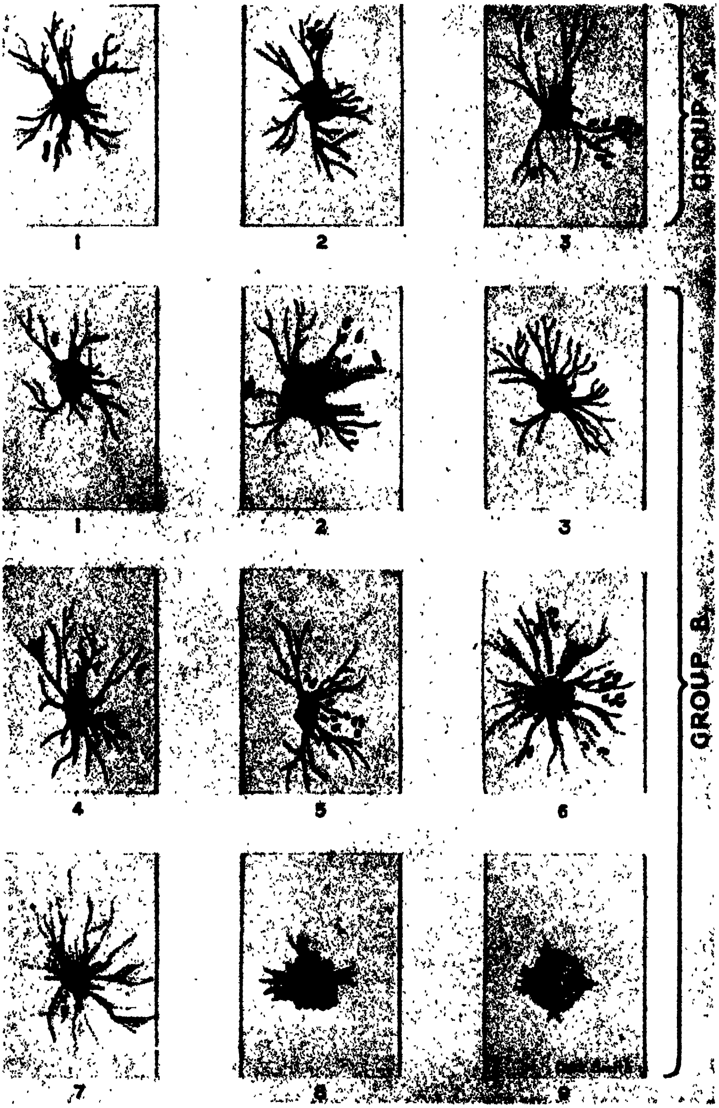
Becher (1924) refers to certain early morphological stages of melaniridosome formation. Such stages exist when isolated iridocytes lie along the margin or over the centre of a melanophore and also when iridocytes are partially or even completely surrounded by dendrites of a melanophore. Early stages of melaniridosomes of this description were noted within normal cutaneous areas of the Bermuda grunt. Inasmuch as they are found in the regenerating experimental wounds described above, support is given to the view that the small chromatic organs known as melaniridosomes are formed by a general and continuous attraction of melanophore and iridocytes to each other, resulting finally in an intimate association.



TEXT-FIG. 3.

A field of iridocytes and newly formed melanophores on the surface of a wound 7 days old, direct light. A B C, margin of wound. Clusters of iridocytes (small white spots) are scattered throughout the field. Newly formed melanophores have become associated with clusters of iridocyte bodies to a varying degree, melanophores X, Y, Z, showing well-advanced melaniridosome formation. Microphotograph.

Becher (1924) points out that the metallic lustre of iridocytes



TEXT-FIG. 4.

changes with the difference of contraction and expansion of the melanophore surrounded by or embedded in these crystalline bodies. The melaniridosome represent, therefore, a grouping of tissues which functions as a minute unit in the colour changes of certain fishes.

SUMMARY.

A description is given of the formation of a simple type of chromatic organ or melaniridosome occurring during the healing of experimental wounds in two species of grunt (Bathystoma aurolineatum and Haemulon sciurus). Newly formed melanophores and iridocytes in the substance of the wound became attracted to each other, and group themselves into permanent masses known as chromatic organs or melaniridosomes. Chromatic organs of this type were observed in later stages of wound healing (6–10 days) when epithelialization and formation of the new corium were well advanced.

Text-fig 4 (*see opposite*).

Association of iridocyte bodies with melanophores to form melaniridosomes diagrammatically illustrated from drawings made of living cells in regenerating wound of Haemulon sciurus (Group A) and Bathystoma aurolineatum (Group B). Structural details of cells are omitted. In both groups A and B, figs. 1–5 are early stages; figs. 6 and 7 more advanced. Iridocytes, represented as small oval or rod-shaped bodies, have accumulated between dendrites or about the body of stippled melanophores. Figs. 8 and 9 represent final stages of melaniridosome formation. Each melanophore, in a phase of contraction, lies embedded in a mass of iridocytes.

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The Early Development of the Nephridia in Amphioxus: Part II, The Paired Nephridia.

By

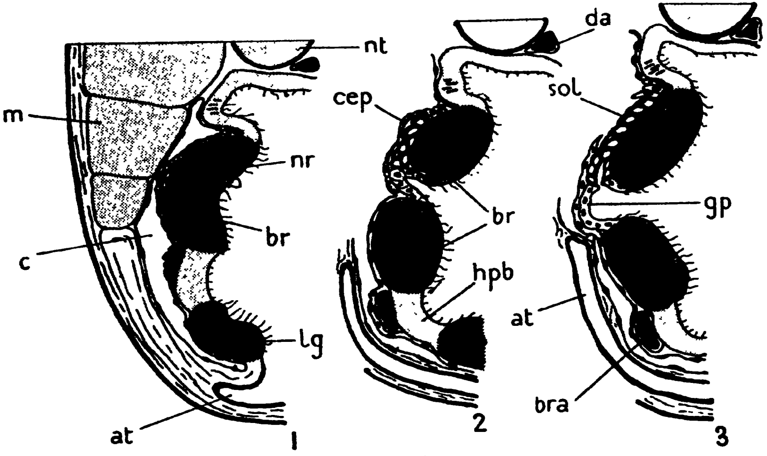
Edwin S. Goodrich, F.R.S.

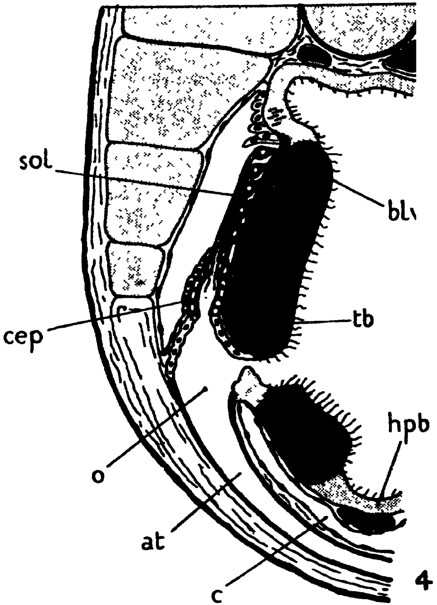
With Plates 37 to 40, and 4 Text-figures.

THE only account we have of the early development of the paired nephridia of the pharyngeal region of Amphioxus is that given by R. Legros (1909) in a preliminary note already referred to in Part I of this contribution (p. 499 of this volume). In the same year a description was given by me (1909) of the nephridia in the larva with about ten gill-slits of the 'primary' or future left series; but at this stage the paired nephridia are already well advanced. They consist of a 'canal' in the form of a rounded sac provided with solenocytes at its inner blind end, and an opening to the exterior at the future dorsal edge of the gill-slit. Since then I have studied in Naples the nephridia related to the developing 'secondary' or future right series of gill-slits in older living larvae undergoing metamorphosis, but was unable to discover their first origin. On these somewhat opaque larvae it was only possible to make out that already, when the gill was represented merely by a rounded thickening of the endodermal wall, the nephridial rudiment could be seen as a small mass of cells on its dorsal margin. By the time the gill-pouch has been formed in the middle of the thickening, and the tongue-bar has begun to grow downwards from its dorsal edge, the nephridial mass has become hollow and solenocytes have grown out from it. Soon the whole organ acquires the structure already described in the 'primary' series (Goodrich, 1909). The details of the development can only be worked out on sections, and it must be confessed that even in sections it is extremely difficult to make out clearly. Unfortunately I was unable to trace the early development of the nephridia belonging to the 'primary' series of gill-slits, since the space in which they develop is so cramped, and its walls become so closely applied to each other, that no distinct image of the origin of the nephridial cells could be obtained. The material used consists of a large number of larvae obtained by me in Naples and a few from Heligoland, fixed and stained in various ways (see Part I, p. 500). I have to thank Prof. J. W. van Wijhe for kindly lending me some sections on which I was able to confirm some of my results.

Legros (1909) begins his account with a description of the development of the 'secondary' or right series of gill-slits already given in outline by Willey (1891), who studied the process in larvae living and mounted whole. Legros points out that the fusion of the wall of the pharynx with the wall of the atrium and the actual perforation of the gill-slit really takes place only towards the end of Willey's stage IV. The first sign of a gill is a rounded thickening of flagellated epithelium on the inner endodermal wall of the pharynx. At the centre of the thickening a few rounded non-flagellated endodermal cells grow outwards to form a small pocket bulging towards the outer or coelomic surface. It is this 'gill-pouch', as it may be called, which Willey mistook for a perforation in his stage II. In stage III the pouch enlarges to form an oval area surrounded by a ring of the thickened epithelium. The rudiment of the atrium, a flattened tube, is still ventral and far away from the gill-rudiment. The tongue-bar next appears in stage IV as a downgrowth from the dorsal region of the ring. It grows freely and internally to the outer wall of the gill-pouch, which extends laterally beyond it. At stage V the ventral tip of the tongue-bar fuses with a small upgrowth from the ventral region of the thickened ring. Meanwhile, the atrial wall grows up on the right towards the gill-pouch and fuses with the lower part of the pouch to form a 'branchial diaphragm'. On the piercing of this thin diaphragm the gill-slit is opened (Text-figs. 1–4).

It follows from this account, which I can fully confirm from my own observations, that, in larvae of stage V when the definitive right slit is completed, the wall of the gill-pouch remains as a layer of endoderm lining the most dorsal region of the atrium and the outer side of the young tongue-bar. It also follows that the nephridium, which has meanwhile been developing between the dorsal part of the gill-thickening and the coelom,





Text-figs. 1–4. Lettering.

Diagrams illustrating the author's account of relation of nephridial rudiment to endoderm and coelomic epithelium as shown in transverse sections of developing right gill-slits of larval Amphioxus. *at*, atrium; *blv*, blood-vessel; *br*, gill-thickening; *bra*, ventral aorta; *c*, coelom; *cep*, coelomic epithelium (with black nuclei); *da*, left dorsal aorta; *gp*, gill-pouch; *hpb*, hypobranchial band; *lg*, edge of left gill-slit; *m*, myomere; *nr*, nephridial rudiment (with white nuclei); *nt*, notochord; *o*, opening of gill-pouch into atrium; *sol*, solenocyte; *tb*, tongue-bar. Text-fig. 1.—Stage II; small nephridial rudiment of few cells covered over by coelomic epithelium. Text-fig. 2.—Stage III; growing nephridial rudiment still covered by stretched coelomic epithelium cells. Text-fig. 3.—Stage IV; young solenocytes uncovered, canal fused with gill-pouch. Text-fig. 4.—Stage V; solenocytes fully formed; atrium has grown up on right side, fused with and opened into endodermal gill-pouch.

acquires an opening first into the gill-pouch and later into that small upper region of the atrium lined with endoderm. Nevertheless, admirable as is the description of the development of these parts given by Legros, it would seem that little importance can be attached to the existence of this small endodermal contribution to the wall of the atrium. The endodermal area appears to be soon encroached upon by atrial epithelium of ectodermal origin, and in later stages the latter appears to have reached the nephridiopore and to cover the outer side of the tongue-bar. The outgrowth of the gill-pouch would seem to be a temporary device for enabling the rudiments of the greatly retarded right series of slits to develop and join the atrial wall. No such extensive outgrowth takes place in connexion with the left slits, though Legros maintains that the nephridiopore is here also first formed in the very small endodermal area, at all events in the case of the more posterior slits. In the adult no difference can be detected between the structure and relations of these various parts on the two sides of the body.

With regard to the development of the nephridia themselves, Legros deals in detail with those belonging to the right series of gill-slits. From the second backwards these slits and their accompanying nephridia develop from before backwards. But the first gill-slit, as shown by Willey (1891) is delayed, and does not appear even as a thickening until stage III. Moreover, it is not subdivided by a tongue-bar. The conditions here appear simpler, and accordingly Legros proceeds to describe the development of the first nephridium. But there is a complication owing to the presence of a 'septum' reaching obliquely downwards and backwards from the roof of the splanchnocoele to the middle of the gill-rudiment. According to Legros, similar intersegmental septa, which appear to be remnants of the original walls between adjacent mesoblastic segments, and correspond in position to the tongue-bars, are small and quickly disappear; but that opposite the first gill-rudiment is better developed and persists longer. He figures it in transverse sections as close to and overhanging the rudiment of the first nephridium. The nephridium itself is said to appear first as a small longitudinal fold of the coelomic epithelium (splanchnopleure) on the outer surface of the young gill-thickening (his fig. 9, p. 142). This fold then bends upwards, closing posteriorly to form the blind end of a short tube opening forwards and upwards into the coelom. The coelomic epithelium cells near the edge of the funnel become converted into solenocytes. The mouth of the funnel then closes by approximation of its outer and inner lips, the solenocyte tubes passing in between them. Meanwhile, the posterior blind end of the canal fuses with the dorsal region of the developing gill-pouch, and finally a pore is pierced leading into the cavity of this pouch. Later, when, as described above, the 'diaphragm' of the gill-slit is broken through, the first nephridium comes to open into the atrium.

Some details and figures are given of the development of the more posterior nephridia which agree with those of the later stages of the first; but there is no figure of the earliest stage in these segments, when the rudiment is supposed to be in the form of an open groove or funnel.

From the above brief summary of Legros' results it is clear that, as in the case of Hatschek's nephridium discussed in Part I of this contribution, he believed that both nephridial canal and solenocytes are derived from coelomic epithelium. A fold of this epithelium is said to give rise to a funnel-shaped tubule, whose internal opening soon closes, and the tubes of the solenocytes are supposed to reach the nephridial lumen in some way which is not fully explained.

The description of my own observations may begin with late stages of the nephridia posterior to the first pair. At stage V of Willey, for instance, the nephridium is seen on the outside of the tongue-bar in the form of a short flattened canal opening into the endodermal dorsal region of the atrium. From its blind dorsal end extend some solenocytes whose cell-bodies rest on the inner wall of the splanchnocoele (epibranchial coelom). While the canal is covered with coelomic epithelium, this layer is absent over the solenocytes (figs. 1–4, Pl. 37, also fig. 8 *a* and *b*, p. 135, of Legros, 1909). The general relations of the parts are drawn in Text-fig. 4, and it will be seen that, though of much smaller size and simpler structure, the nephridium at this stage resembles in every essential and in its relation to surrounding tissues that of the adult (Goodrich, 1909).

The nephridium in stage IV of Willey (fig. 5, Pl. 37, and Text-fig. 3) differs little from that just described, but is of some interest because it is the oldest in which Legros figures distinctly what he holds to be a narrow remnant of the alleged funnel opening (his fig. 5, p. 131). My fig. 5, Pl. 37, shows a section through the nephridial rudiment with a pore into the endodermal gill-pouch, but with no internal opening into the coelom and a covering of coelomic epithelium.

Legros figures a nephridium of stage III of Willey (his fig. 4 *b*, p. 130) with the nephridiopore already open, and with a row of crowded dark cells, young solenocytes, extending dorsalwards from the small canal. My fig. 6, Pl. 37, gives a general view of a transverse section of a Naples larva of this stage, while fig. 7 A, B, C, Pl. 37, shows three consecutive sections on a larger scale through a Heligoland larva at a similar stage, but slightly less advanced. Here the very short canal is seen opening into the gill-pouch at the point *np*, fig. 7 C, and a number of cells are seen to spread out from its blind dorsal end in the two more anterior sections (see also Text-fig. 2).

Larvae at stage II of Willey have very small incipient gill-pouches, and Legros (his figs. 2 and 3, p. 129) figures the nephridial rudiment as consisting of a few clear cells passing dorsally from the gill-pouch into a rather confused mass of darker cells. This is the earliest stage he figures of the developing posterior nephridia. My figs. 8 A, B, C, and 9 and 10 A, B, Pl. 37, are of about the same stage. In the last two figures the sac-like canal is shown to be not yet fully formed, and the nephridial rudiment appears only as a row of a few cells extending for a considerable distance dorsally from the developing gill-pouch. The more dorsal of these cells are, no doubt, young solenocytes, and the more ventral represent the rudiment of the canal. No clear distinction can be made out between them at this stage. Fig. 8, Pl. 37, on the other hand, shows a more advanced nephridium. Here the canal has distinct wall and lumen, but the nephridiopore is not yet formed owing to the very undeveloped state of the gill-pouch. Some of the solenocytes, however, appear to be joined to the canal by a process which may be a developing tube. It will be noticed that an artificial space, *a*, due to the defective fixation of this specimen, separates the solenocytes from the endodermal gill-thickening.

So far only characteristic sections through typical examples of stages II–V, corresponding to those figured by Legros, have been described.

We must now examine the evidence more in detail in order, if possible, to decide on three important points of difference between my own interpretation and that of Legros. These points are: (1) the first origin of the nephridium; (2) the origin of the solenocytes; and (3) the relation of the nephridial rudiment to the coelomic epithelium.

It will be best to deal with the third point first. In the adult nephridium (Goodrich, 1909), although the canal is covered by coelomic epithelium, and is therefore 'retroperitoneal' in position, the 'solenocyte field' (that area over which the solenocytes spread) is uncovered, and the tubes of the solenocytes are exposed to the coelomic fluid. Therefore, I concluded that the coelomic epithelium, which must have originally covered the whole excretory organ, had become interrupted or broken through and pushed aside by the growing solenocytes. That this is the correct interpretation is, I believe, borne out by a study of the development.

For this purpose stages II–IV must be re-examined, and earlier stages as well. Moreover, since there is no hard and fast line between the various stages, and since new gill-slits appear at the hinder end of the series in these larvae, the gaps between typical stages can be filled in from older larvae.

It has already been mentioned that in stage V the fully developed nephridia resemble those of the adult in that the solenocytes are uncovered. Usually in stage IV the solenocyte tubes are not yet fully differentiated, being represented by cytoplasmic strands, and their cell-bodies are more closely set together than in later stages. It is often scarcely possible to distinguish at the edge of the field between the young solenocytes and the cells of the coelomic epithelium, which here are also closely packed. But the epithelium is already interrupted over the field, although some coelomic cells appear occasionally to overlap its edge.

It is not till we reach the earlier stage III that we find constant traces of a more or less complete covering of coelomic epithelium over the whole nephridial rudiment, solenocytes as well as canal. Concerning the coelomic epithelium in general, it may here be mentioned that it varies much in character. In most places it is thin and composed of flattened cells with flattened nuclei set very far apart; but in others, as for instance on the main branchial artery (fig. 12, Pl. 38, *bra*), it may be formed of irregularly shaped cells with oval or rounded nuclei. On the young developing gill-thickenings the epithelium becomes in most places quite thick with closely set more or less cubical cells (fig. 12, *cep*, Pl. 38.) This type of epithelium seems to occur in the more actively growing parts, and mostly flattens out later.

In the section drawn in fig. 8, Pl. 37, it is doubtful whether the coelomic epithelium covers any of the young solenocytes, but over the canal region it is obvious; and in fig. 9, Pl. 37, it can be seen passing over the few solenocytes appearing in this section much as in fig. 5, Pl. 37. In fig. 10 B, Pl. 37, of a section cutting through the base of the canal region where it joins the growing gill-pouch, the coelomic epithelium is well shown and can be traced farther forward in fig. 10 A, Pl. 37, as a film of protoplasm covering the closely set solenocyte cells. The epithelium can also be seen in fig. 11 A, Pl. 37, stretching over the whole nephridial rudiment, and in the next section (fig. 11 B) covers a few young solenocytes. The three consecutive sections drawn in fig. 7 A, B, and C, Pl. 37, show clearly how the coelomic epithelium covers both canal and solenocytes in a more typical stage III.

At stage II of Willey, when the gill-pouch has scarcely begun to develop, the coelomic epithelium can usually clearly be seen covering the whole nephridial rudiment. It is well shown in fig. 13 A and B, Pl. 38, and can again be traced in fig. 14 A and B, Pl. 38, of a rather less developed gill-thickening. In still less advanced larvae of this stage, or in sections of posterior gill-thickenings at a corresponding stage of development, the nephridial rudiment, now very small, is seen to be completely covered (figs. 15, and 16 A and B, Pl. 38).

Coming to still earlier stages in the development of a gill-slit, when the endodermal thickening has scarcely any sign of gill-pouch beyond a slight depression in its centre (figs. 15–18, Pl. 38), we find only a few nephridial cells lodged between endoderm and overlying coelomic epithelium. Even here, however, the latter may be stretched out in places into a thin protoplasmic layer.

Finally, in yet younger stages, only two or three, or even one cell can be found between endoderm and coelomic epithelium in the appropriate place (figs. 19–21, Pl. 38; 22, Pl. 39).

It is important to notice that in these earlier stages the nephridial rudiment is not continuous with the endoderm, but separated from it by a thin well-defined basement membrane. Fig. 14 B, Pl. 38, shows the beginning of the breaking down of this membrane preparatory to the fusion of the nephridial cells with the endoderm at the place where the nephridiopore will eventually be formed. In spite of the perfect continuity of the two tissues established later, there can be no doubt, as already maintained by Legros (1909), that the nephridial cells are not derived from the endoderm.

The general result of my observations on the early stages described above is that the nephridial rudiment lies at first between the endoderm within and the coelomic epithelium without; and that, as the rudiment enlarges and spreads, the coelomic epithelium becomes gradually stretched and finally ruptured over the solenocyte-field while remaining unbroken over the developing canal. Certain sections seem to show that whole cells overlying the solenocytes may be thrown off. Of any folding of the coelomic epithelium to give rise to an open funnel later closed to form the canal, as described by Legros, no trace could be found (Text-figs. 1–4).

But, since Legros for the evidence of the existence of such a fold relied chiefly on his study of the nephridium belonging to the first right gill-slit, it is necessary now to examine in detail the case of this slit which develops later than the more posterior ones.

The first gill-thickening appears in stage III of Willey, when the club-shaped gland is degenerating. Unfortunately, Legros gives no details about its relation to the gland. My own specimens show that at this stage, when the gland is undergoing disintegration and phagocytosis or has just disappeared, there is usually a somewhat confused mass of cells obscuring the processes of development taking place in this region. By the time the glandular part of the club-shaped gland has been destroyed and its dorsal part and pharyngeal opening have disappeared, the first gill-thickening has usually considerably advanced in development, and it is extremely difficult to make out the origin of the nephridium and of the 'septum' which overhangs it (see p. 658).

When, in stage IV, this septum is well developed and clearly marked it appears in transverse sections as a fold extending outwards into the coelom from the splanchnic wall at the dorsal limit of the gill-thickening (figs. 24–8, Pls. 39 and 40). It is composed of a thin lamella springing from the basement membrane, and covered on both sides by coelomic epithelium. It dwindles away in front, rising up to join the dorsal wall of the coelom. Posteriorly it spreads downwards, finally merging into the basement membrane of the gill-thickening at the posterior edge of the developing gill-pouch. Thus the septum about this stage may often have a small 'cul-de-sac' at its posterior end, and spreads forward over the nephridial rudiment. It usually presses so closely on to the latter as to render it very difficult to distinguish the true limits of the rudiment, and whether the nephridial cells are covered over with coelomic epithelium or not (figs. 24–8, Pls. 39 and 40). Nevertheless, in some of the series of sections it can be seen that in general structure and relations the rudiment of the first nephridium develops in the same way as do rudiments in the more posterior segments described above. For instance, figs. 24 and 25, Pl. 39, show a stage of development equivalent to that of posterior nephridia in figs. 10 and 11, Pl. 37; figs. 26 and 27, Pl. 39, correspond to figs. 9 and 14, Pls. 37 and 38; and fig. 28, Pl. 40, to fig. 15, Pl. 38. In the last case (fig. 28), the nephridial rudiment consists of only a few cells not yet continuous with the endoderm.

Earlier stages than this are still more difficult to interpret owing to the fact that the club-shaped gland is still present, large, and more or less closely applied to the cells covering the outer surface of the incipient first gill-thickening. I have not attempted to trace in full detail the origin and development of the septum, but have often found it well formed in stage III and even sometimes in stage II. It appears to be related to the club-shaped gland, covering its posterior surface, and when the gland degenerates the septum remains adhering to the developing first gill-thickening. Figs. 29–31, Pl. 40, illustrate these stages. It will be noticed that it is very difficult to recognize any nephridial rudiment in these sections. Some of the closely packed cells below the septum in the most advanced of these larvae (fig. 29, *nr*) probably belong to the nephridium, but in the less developed larvae (figs. 30 and 31), no cells could be identified with certainty as belonging to the nephridial rudiment, though the cells marked *nn* in the figures may possibly represent it.

My general conclusion with regard to the first right nephridium is that, like the more posterior nephridia, it develops from cells situated at first between endoderm and mesoderm. Legros gives good figures showing the relation of the septum to the nephridium in stage IV (his fig. 12, p. 143, and fig. 13, p. 144, 1909) which agree with my observations, excepting of course for the alleged internal opening of the canal. It is in the interpretation of the earlier stages that we differ radically.

He gives two figures of a larva at stage IV where the nephridial rudiment has not yet fused with the endoderm, and is supposed to be in the form of a simple funnel, blind behind, but opening into the coelom in front (his figs. 10 *a* and 10 *b*, p. 142, 1909). The first of these shows no opening since the section is said to pass immediately behind it; the second shows only the blind end. Now, it will be noticed that, whereas a septum appears in his figures of an earlier and of two later stages, no septum is drawn in these particular figs. 10 *a* and 10 *b*, where it should undoubtedly be visible. It seems probable, then, that what Legros took to be a funnel-like stage of the nephridium was really the posterior region of the septum itself which, as described above, may form a shallow 'cul-de-sac'.

If this interpretation of these figures of Legros be correct, it follows that the chief evidence he brought forward to support his view that the nephridium develops from a diverticulum of the coelomic epithelium collapses. Of the earlier stage III, where the nephridium is supposed to be a mere fold of the coelomic epithelium, he gives only one unconvincing figure (his fig. 9, p. 142, 1909). Here it seems doubtful whether the part marked *spt* (above the peripharyngeal band instead of below it) is really the septum at all; while the alleged rudiment of the nephridium might well be the incipient septum. Many of my sections show similar folds.

My observations on the development of the nephridium belonging to the first right gill-slit afford no evidence in support of the interpretation put forth by Legros. This first nephridium seems to develop like the more posterior ones from special cells, not from a funnel folded off from the coelomic epithelium.

We come now to the second point to be discussed (see p. 661)—the first origin of the nephridium. Legros has quite rightly maintained that the nephridial rudiment is not derived from the endoderm, and that its connexion with the gill-pouch is secondary. According to my own results, described above, a nephridium of the right side can be traced in early stages to a small group of cells lying between the coelomic epithelium and the incipient endodermal gill-thickening (fig. 22, Pl. 39). We might expect to be able to trace back this group even to a single cell in still earlier stages. Certainly, at a stage when the gill-thickening is only very slightly marked or scarcely formed at all, one can generally find near its centre a rather conspicuous cell lodged between the endoderm and coelomic epithelium (fig. 23, Pl. 39). Although it is difficult to establish beyond doubt the nature of such a single cell, it is very probable that it represents the rudiment of a nephridium and is in fact a nephroblast.

We might expect to find such isolated nephroblasts in still earlier stages. With this object in view I have searched through series of sections of young larvae at stages before the appearance of any gill-thickenings at all on the right side of the pharynx, and have sometimes succeeded in discovering at appropriate intervals single cells between endoderm and coelomic epithelium. Nevertheless, these observations cannot be held to be sufficiently definite to afford convincing evidence of the existence of nephroblasts at this early stage.

From our knowledge of the development of true nephridia in other groups, however, its seems extremely probable that such nephroblasts are set apart at the growing hinder end of the embryo Amphioxus when the tissues are still undifferentiated, and become distributed in the appropriate positions as the young larva grows in length. However this may be, the important point, which it is claimed has been established in this paper, is that the nephridium is not formed later by a folding off of the coelomic epithelium.

The third and last point to be decided must now be discussed —the origin of the solenocytes. In spite of the statement of Legros (1909) that these cells are derived from the coelomic epithelium (see p. 659), the most careful study of my preparations under the highest available powers of the microscope has convinced me that his conclusion is not correct. From my description given above of the development of the nephridial rudiment it will be gathered that in early stages it is in the form of a continuous plate of cells situated at first below the coelomic epithelium. Later the more peripheral and dorsal cells of this plate become distinguishable as young solenocytes connected with the developing canal by protoplasmic strands, rudiments of the tubes. Later on still, as the cells spread over the expanding solenocyte field, the tubes lengthen and become more definite. It is true that, in these early stages, it is often very difficult to distinguish at the edge of the field between cells which are to become solenocytes and cells which belong to the epithelium. Nevertheless, no good evidence could be found in my preparations that the latter were ever converted into the former. But it must be admitted that many gaps in the detailed history of the differentiation of the solenocytes remain to be filled. This task is rendered more difficult owing not only to the small size of these cells, but also owing to the strange absence of dividing nuclei among them. Only rarely have divisions been met among the cells of the canal. The solenocytes seem to be formed by the drawing away of peripheral cells from the canal rudiment, and once drawn away they possibly never divide again. On the other hand, the development of the nephridium in the larva must undoubtedly be very slow, and it may also be suspected that nuclear division takes place in it mostly at night and hence usually escapes notice.

My conclusion, founded on the repeated and careful study of a large number of series of sections of various stages, is that the nephridia of Amphioxus are genuine protonephridia strictly homologous with the protonephridia of such Invertebrata Triploblastica as the Platyhelmia, Nemertina, and Annelida, and indeed with true nephridia in general. The extraordinarily close resemblance between the nephridia of Amphioxus and the solenocyte-bearing nephridia of certain Polychaeta (Goodrich, 1902) already rendered this conclusion in the highest degree probable even before their development was known. But some doubts as to the homology may well have arisen when Legros' papers appeared. If so, these doubts may now be set at rest, since the evidence here given concerning the development of these organs in Amphioxus is in agreement with what is known of their development in other Triploblastica. It follows that, as maintained by me since 1902, the protonephridia of Amphioxus can have nothing to do with the genital and excretory ducts of the Vertebrata Craniata, which are clearly of the nature of coelomoducts.

Summary of Parts I and II.

Part I of this contribution (p. 499 of this volume) deals with the unpaired anterior nephridium of Amphioxus. It opens dorsally into the pharynx, runs along the left aorta to end blindly in front, and is known as Hatschek's nephridium.

This nephridium was described by Legros (1910) as developing, in the young larva before the opening of the mouth, from a funnel-shaped backward growth of the posterior wall of the coelomic cavity of segment 2. Its posterior end was said to acquire a pore into the pharynx, while the open anterior end became closed and connected with solenocytes developed from neighbouring cells of the coelomic epithelium. These conclusions have not been confirmed.

According to my own observations, at a stage before the buccal diverticulum has even appeared, the rudiment of the nephridium can first be identified as one or two cells between the posterior wall of segment 2 and the anterior wall of segment 3. By the time the mouth has been pierced, the rudiment appears as a group of cells between these two segments. At first solid and independent, it later acquires a lumen and opens into the pharynx through the endoderm. Cells grow from the blind internal end of this rudiment towards the cavity of segment 2, the coelomic epithelium being now broken through. That portion of the coelomic cavity which lies between the second myotome above and the splanchnocoele below becomes separated off as a closed chamber (as also shown by Legros), and into it grows Hatschek's nephridium. Later, the chamber becomes reduced and broken up into remnants into which project the solenocytes of the adult organ.

Canal and solenocytes are derived from the same rudiment. At no stage could an open funnel be found. The first origin of the cell which gives rise to the rudiment could not be traced, but it seems to come neither from endoderm nor from mesoderm, nor yet from the ectoderm of the anterior region of the embryo at the stages of development investigated.

Part II of this contribution deals with the paired nephridia related to the right gill-slits which develop in the larvae of Amphioxus during stages II–V of Willey.

The description given by Legros (1909) of the development of the right gill-thickening of the pharyngeal wall, of the outgrowth of an endodermal gill-pouch, of the formation of a gill-membrane which is pierced to afford communication with the ectodermal atrium so that the definitive gill-slit opens at first into a dorsal region of the atrium lined with endoderm, is fully confirmed. Also that the nephridial canal comes to open at first into the endodermal gill-pouch before the gill-membrane is pierced, as he described. But there is reason to believe that later the ectodermal atrial wall encroaches on the small endodermal portion, so that in the adult the right nephridia open approximately at the junction of the two layers as on the left side.

On the other hand, no evidence could be found for the view of Legros that the nephridium is developed from a fold of coelomic epithelium giving rise to the canal, open to the coelom at first by a funnel which closes later, and that cells of the coelomic epithelium are converted into solenocytes.

According to my own observations here described, the rudiment of the right nephridium occurs in stage III as a small group of cells between the endodermal wall of the pharynx (gill-thickening) on the inside and the coelomic epithelium on the outside. These cells increase forming a plate over which the coelomic epithelium is stretched then ruptured, so that the growing solenocytes become exposed to the coelomic fluid. The more dorsal cells of the plate become the solenocytes, and spread over the expanding solenocyte field, their tubes being correspondingly lengthened. The coelomic epithelium remains covering the more ventral cells of the plate which form the canal. This canal fuses with the dorsal wall of the gill-pouch at a point where the nephridiopore comes to open. By stage V the nephridium has acquired essentially the structure of the adult organ. Both nephridial canal and solenocytes develop from the same rudiment lying at first between endoderm and mesoderm. At no stage is there an internal opening.

It is further maintained that the nephridium of the first gill-slit arises in the same way as the posterior ones, but that its development is complicated by the presence of a septum related to the club-shaped gland.

The nephridial rudiment of a few cells appears to arise in earlier stages (stages II and III of Willey) from a single cell in the same position; but this could not be determined with certainty. It may, however, be supposed that such nephroblasts exist, are differentiated at the posterior end of the embryo, and are distributed later as the segments develop and the embryo lengthens. They must presumably be of ectodermal or of ecto-mesodermal origin. The nephroblast giving rise to Hatschek's nephridium may be supposed to be of similar origin.

This conclusion is in agreement with the view that the nephridia of Amphioxus are true protonephridia, homologous with the protonephridia of the Invertebrata; but not homologous with the excretory tubules of the Vertebrata Craniata, which are derived from coelomoducts.

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EXPLANATION OF PLATES 37 TO 40.

LETTERING.

a, artificial space; *at*, atrium; *blv*, blood-vessel; *br*, right gill-thickening; *bra*, branchial artery = ventral aorta; *c*, coelom; *cep*, coelomic epithelium; *csg*, club-shaped gland; *da*, left dorsal aorta; *end*, endostyle; *ep*, epidermis; *epc*, epibranchial or lateral dorsal coelom; *gm*, gill-membrane; *gp*, endodermal gill-pouch; *go*, opening of gill-pouch pierced through gill-membrane into atrium; *hpc*, hypobranchial coelom; *hpb*, midventral hypobranchial band; *lgo*, left gill opening into atrium; *m*, myomere; *n*, nephridium; *nn*, nephridial cell?; *np*, nephridiopore; *nr*, nephridial rudiment; *nt*, notochord; *ph*, wall of pharynx; *sol*, solenocyte; *sp*, septum belonging to first right gill; *tb*, developing tongue-bar.

All the figures are transverse sections drawn with a camera. The magnification of figs. 1, 2, and 6 is given by the scale attached to fig. 1.

The magnification of all the other figures is the same, and given by the scale on each plate.

PLATE 37.

Fig. 1.—Section of larva from Heligoland, stage V, through eighth right slit, and opening of nephridium. Endodermal pouch of slit not yet open into atrium on right side.

Fig. 2.—Section through seventh right slit of same larva. Branchial membrane pierced, and right slit open. Nephridial pore cut through.

Fig. 3 A and B. Two consecutive sections through tongue-bar of a gill-slit about to be pierced, stage V. Ventral connexion of tongue-bar not yet formed. In A branchial membrane complete; nephridium opening into endodermal gill-pouch. In B branchial membrane interrupted.

Fig. 4.—Section through anterior region of a gill-thickening (only a portion of which is shown) showing uncovered young solenocytes. Stage V.

Fig. 5.—Section through a nephridium opening into a gill-pouch. The whole nephridial rudiment is at all events partly covered by coelomic epithelium. Stage IV.

Fig. 6.—Transverse section through fourth right gill-thickening of stage III. Developing gill-pouch not yet opening into atrium.

Fig. 7 A, B, and C.—Three consecutive sections of third gill-thickening of stage III from Heligoland. A, most anterior, and B show nephridial rudiment still covered by coelomic epithelium. C cuts through nephridial opening into small gill-pouch.

Fig. 8 A, B, and C.—A, most anterior section, shows some nephridial cells covered by coelomic epithelium. B, next posterior section; developing solenocytes torn away from branchial thickening leaving artificial gap; nephridial rudiment forming closed vesicle continuous with outgrowing gill-pouch. C, section next but one posterior showing one distinct nephridial cell below coelomic epithelium. Stage II.

Fig. 9.—Section through more posterior gill-thickening of same larva as in fig. 7 showing coelomic epithelium stretched over nephridial rudiment which is continuous with endodermal cells of outgrowing gill-pouch.

Fig. 10 A and B.—Two consecutive sections of stage III, A anterior, B posterior. Nephridial rudiment still covered by coelomic epithelium.

Fig. 11 A and B.—Two consecutive sections of next more posterior gill-thickening of same specimen as in fig. 10. The more anterior section, A, shows only three nephridial cells below the coelomic epithelium.

PLATE 38.

Fig. 12.—Section through anterior region of gill-thickening to show disposition of coelomic epithelium, &c. Stage III.

Fig. 13 A, B, and C.—Most posterior section A and next anterior B show nephridial rudiment covered by coelomic epithelium and just continuous with outgrowing gill-pouch. Section C, next but one anterior, shows coelomic epithelium only. Stage II, second gill-thickening.

Fig. 14 A and B.—Consecutive sections through a gill-thickening with future gill-pouch marked by mere depression. In posterior section . B nephridial rudiment not yet quite continuous with endoderm. Both A and B show coelomic epithelium stretched over entire nephridial rudiment. Stages II–III.

Fig. 15.—Section through middle of very young slightly marked eighth gill-thickening, stage III, showing nephridial rudiment of a few cells between coelomic epithelium and endoderm.

Fig. 16 A and B.—Portion of sections of stage III through gill-thickening with beginning of gill-pouch marked by mere pit on its inner surface. A, anterior section showing nephridial rudiment of a few cells below coelomic epithelium. B, next section but one more posterior, nephridial rudiment still visible below coelomic epithelium.

Fig. 17 A and B.—Two consecutive sections through second gill-thickening, stage III. Nephridial rudiment a few cells between coelomic epithelium and endoderm.

Fig. 18 A and B.—Two consecutive sections, A anterior, through seventh gill-thickening, stages II–III. Nephridial rudiment a few cells between endoderm and coelomic epithelium which is stretched and almost broken.

Fig. 19 A–D.—Four consecutive sections of very young third gill-thickening only partly drawn, stage II. Nephridial rudiment of few cells between endoderm and attenuated coelomic epithelium.

Fig. 20 A, B, and C.—Three consecutive sections, A most anterior, through middle portion of gill-thickening of larva from Heligoland, stages II–III. Beginning of gill-pouch only slightly marked in A and B. Small group of cells below coelomic epithelium seems to represent rudiment of nephridium.

Fig. 21 A, B, and C.—Three consecutive sections of second gill-thickening, stage III. Small nephridial rudiment of few cells covered by coelomic epithelium.

PLATE 39.

Fig. 22 A and B.—Two consecutive sections of third gill-thickening of stage II from Heligoland. Small nephridial rudiment of a few cells between coelomic epithelium and endoderm.

Fig. 23.—Section through a posterior gill-thickening scarcely marked, stage II. A single cell, *cn*, may represent nephridial rudiment.

FIG. 24 A–E.—Five consecutive figures, A most posterior and E most anterior, through first gill-thickening, stage IV. A, B, and C show septum covering young nephridial rudiment. E shows septum alone.

Fig. 25 A, B, C, D.—Four consecutive sections through the first gill-thickening, stage III. Septum, not clearly defined in this series, appears in most anterior section A, and passes backwards into basement membrane of gill-thickening in most posterior section D. Nephridial rudiment just becoming continuous with endodermal gill-pouch. Two dorsal cells labelled *cep* possibly belong not to coelomic epithelium but to nephridial rudiment.

FIG. 26 A–E.—Five consecutive sections, A most posterior and E most anterior, through first gill-thickening, stage IV. Gill-pouch beginning to form. Relation of septum to nephridial rudiment in A, B, and C; septum alone in D and E.

Fig. 27 A, B, C, D, E.—Five sections of first gill-thickening, stages IV–V. A, most anterior section in front of gill-thickening shows septum. Nephridial rudiment appears in B, three sections more posterior, and in next two sections C and D. In E septum joins basement membrane of gill-rudiment.

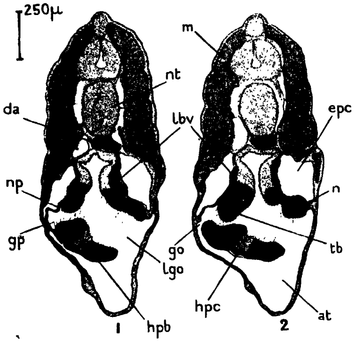
PLATE 40.

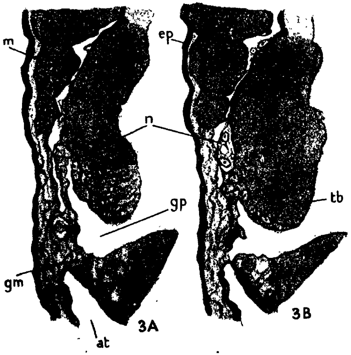
Fig. 28 A–D.—Four consecutive sections through first gill-thickening, stage IV. In D, most anterior section, septum appears dorsally overhanging nephridial rudiment, which is covered by coelomic epithelium. In most posterior section, A, septum has merged with basement membrane of outgrowing gill-pouch.

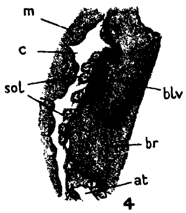
Fig. 29 A, B, C.—Three consecutive sections showing septum attached to very young first gill-thickening, stage III. A, most anterior section, shaves through posterior part of degenerating club-shaped gland. Nephridial rudiment appears as a plate of cells of ill-defined limits. C, most posterior section, passes behind club-shaped gland.

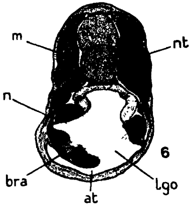
Fig. 30 A, B, C.—Three alternate sections through region of first gill-thickening of very young stage III. Thickening, scarcely marked, appears in most posterior section C, which shaves through posterior wall of still well-developed club-shaped gland. One or two cells, *nn*, may represent very young nephridial rudiment.

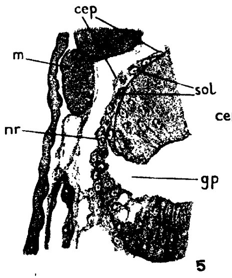
Fig. 31 A, B, C.—Three alternate sections through region of first gill, stages II–III, before appearance of gill-thickening. A, most anterior section, passes through, and C, most posterior, passes behind club-shaped gland. Traces of septum are visible. No distinct nephridial rudiment can be seen unless it be represented by the cells *nn*.

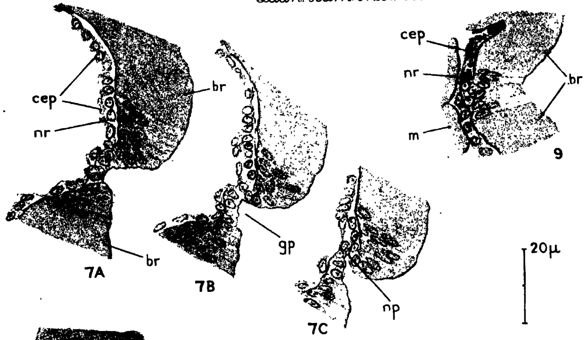


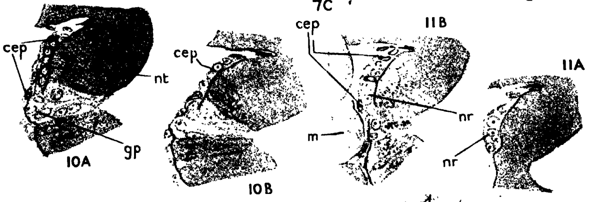


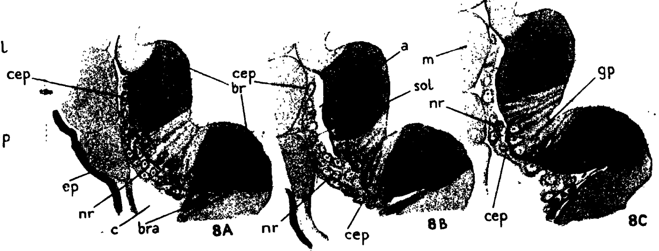












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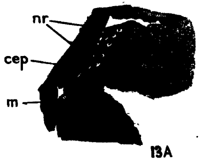

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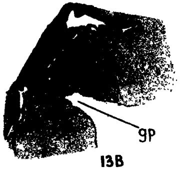

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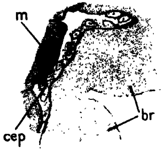

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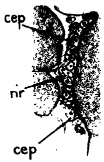

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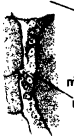

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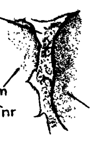

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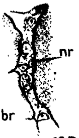
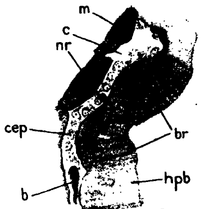


19D



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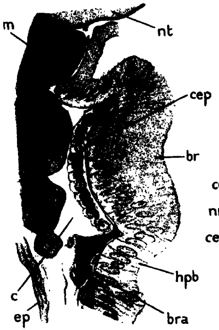

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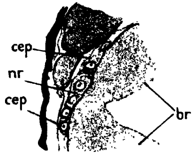

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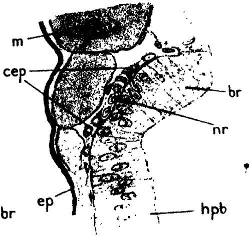

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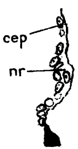

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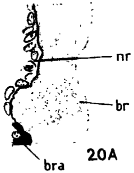

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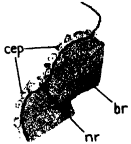

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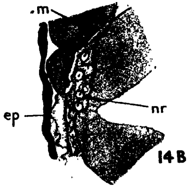

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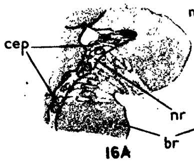

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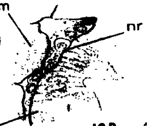

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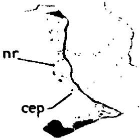

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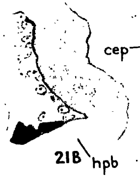

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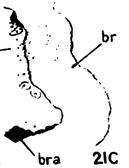

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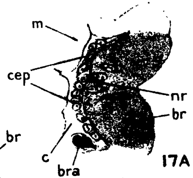

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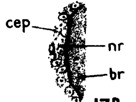

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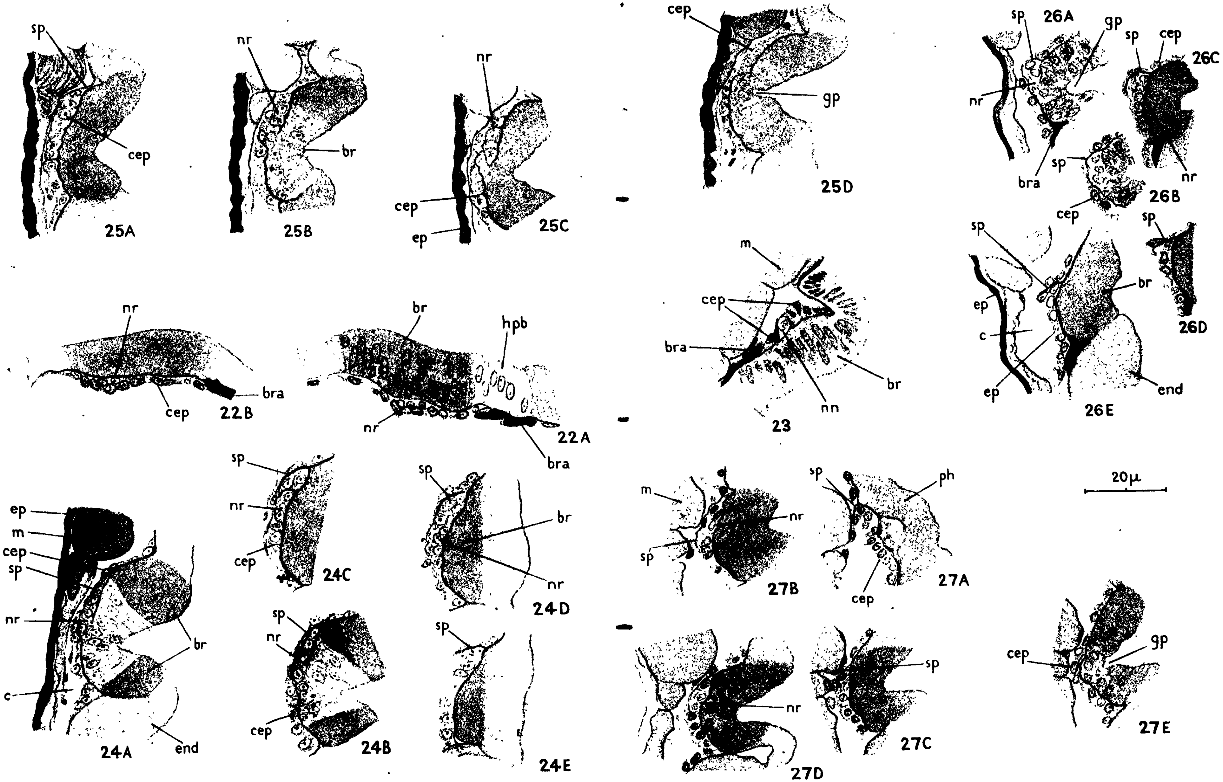

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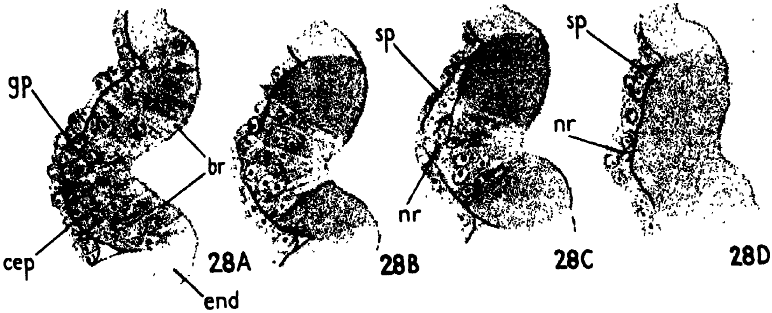

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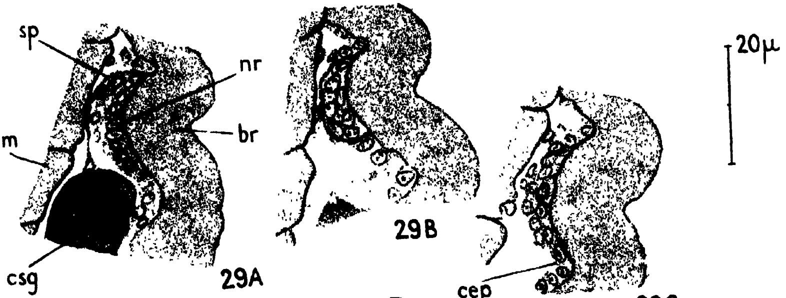

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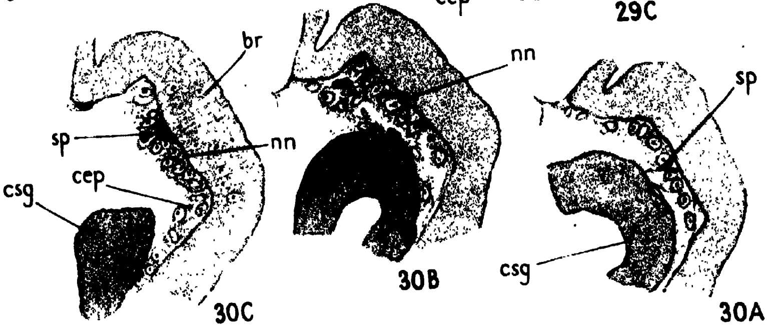

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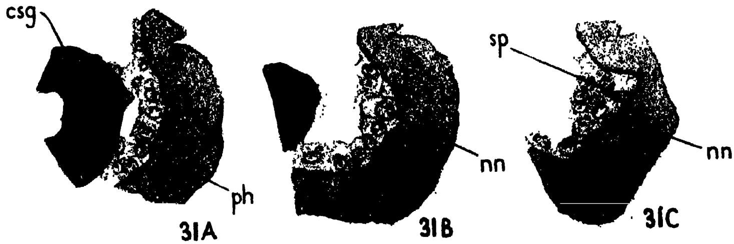

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